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Benzo-Indolic Squaraine Dyes with Large Two-photon Absorption Cross Section

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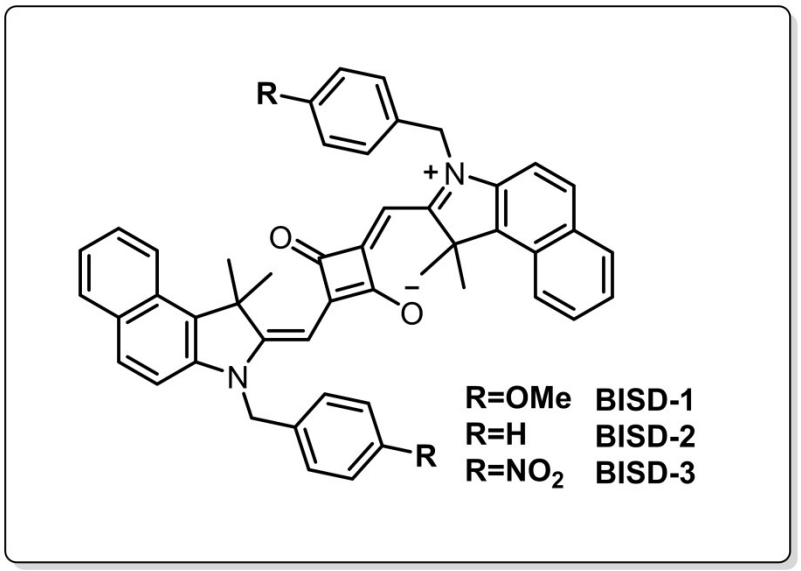
1. Molecule synthesis and characterization

All the reagents and materials were purchased from J&K Chemical Co. (China), and are of analytical grade. The solvents were purified by the standard procedures. NMR spectra were recorded on a INOVA 600MHz spectrometer at room temperature. The chemical shifts (δ) are reported in parts per million and the TMS was used as an internal standard, Mass spectra were obtained on Esquire6000 Bruker Daltonics iron trap mass spectrometer. Elemental analysis was carried out using vario EL cube elemental analyzer.

General Process for the synthesis[1, 2]:

1,1,2-Trimethyl-1H-benzo[e]indole (0.050 mol,), benzyl bromide derivatives (0.075 mol) and acetonitrile (50 mL) were mixed in a 100 mL Schlenk tube and brought to react at 80°C for 8 h. The reaction mixture was chilled below 40°C and then it was poured into diethyl ether (50 L). The precipitate was filtered and washed with cold diethyl ether several times. The residue was dissolved in dichloromethane (20 mL) and crystallized by the addition of diethyl ether (50 L). Repeating three time to get pale yellow powder like solid which were filtered and dried.

In a Dean-Stark apparatus 1,1,2-Trimethyl-1H-benzo[e]indole derivatives (8.2 mmol), squaric acid (4 mmol), toluene 20 mL), 1-butanol (20 mL) were combined and heated to reflux for 8 h. Reaction mixture readily turns deep green. Then the reaction mixture was concentrated in vacuo. All volatiles were removed by vacuum distillation and and the residue was purified by column chromatography on silica gel (DCM/Methanol = 20/1) to give compound BIS-1, 2 and 3.



BIS-1:

^{1}H NMR (600M, DCM-d_2) 2.01 (s, 12H), 3.79 (s, 6H), 5.33 (s, 4H), 5.48 (s, 2H), 7.10 – 7.08 (m, 4H), 7.19 – 7.15 (m, 4H), 7.40-7.58 (m, 6H), 7.62-7.90 (m, 4H), 8.26 (d, J = 9.8 Hz, 2H);
MS (ESI+) 677.3 $[M+1]^+$;

Elemental analysis (%) calculated for $C_{50}H_{44}N_2O_4$: C, 81.50; H, 6.02; N, 3.80; found C, 81.47; 6.04; N, 3.78.

BIS-2:

^{1}H NMR (600M, DCM-d_2) 2.12 (s, 12H), 5.53 (s, 4H), 5.55 (s, 2H), 7.33-7.41 (m, 10H), 7.45-7.62 (m, 6H), 7.65 (dd, J_1 = 8.9 Hz, J_2=8.7 Hz, 2H), 7.96 (d, J = 9.7 Hz, 2H), 8.27 (d, J = 10.2 Hz, 2H);

MS (ESI+) 677.3 $[M+1]^+$;

Elemental analysis (%) calculated for $C_{48}H_{40}N_2O_2$: C, 85.18; H, 5.96; N, 4.14; O, 8.68; found C, 85.20; 5.99; N, 4.12

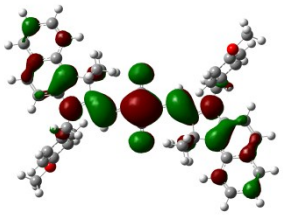
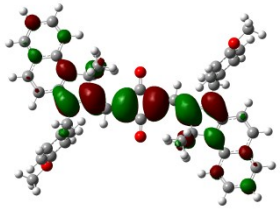
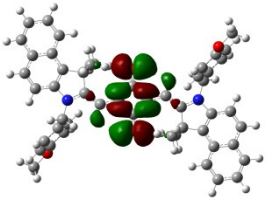
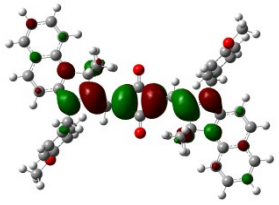
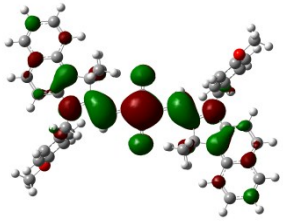
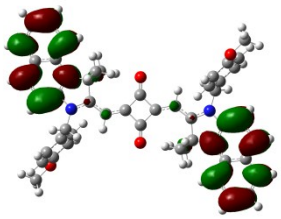
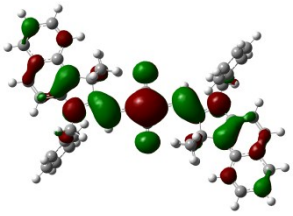
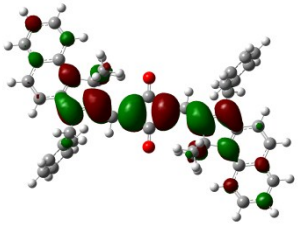
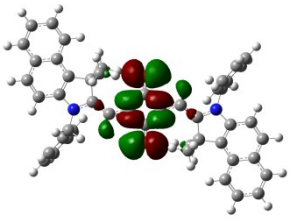
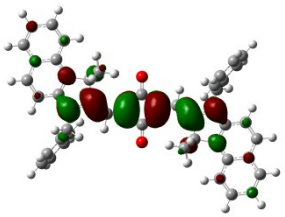
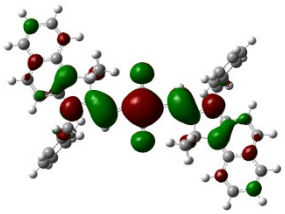
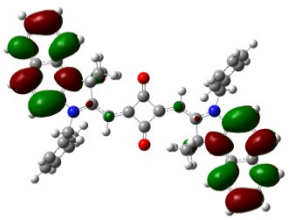
BIS-3:

^{1}H NMR (600M, DCM-d_2) 2.04 (s, 12H), 5.55 (s, 4H), 5.57 (s, 2H), 7.54-7.71 (m, 12H), 7.96 (d, J = 9.7 Hz, 2H), 8.24-8.27 (m, 6H);

MS (ESI+) 767.3 $[M+1]^+$;

Elemental analysis (%) calculated for $C_{48}H_{38}N_4O_6$: C, 75.18; H, 4.99; N, 7.31; found C, C, 75.14; H, 5.02; N, 7.29.

2. The natural transition orbitals (NTOs) of the S_1, S2 and S_3 states

Molecules		HOLE	Particle
BIS-1	|1> W=1.01230 f= 1.5598 2.1482 eV		
	|2> w= 0.99975 f= 0.0000 2.4087eV		
	|3> w=0.93299 f= 0.0000 2.8012 eV		
BIS-2	|1> w= 1.01287 f= 1.5389 2.1518 eV		
	|2> w= 0.99976 f= 0.0000 2.3990 eV		
	|3> w= 0.91526 f= 0.0000 2.8160 eV		

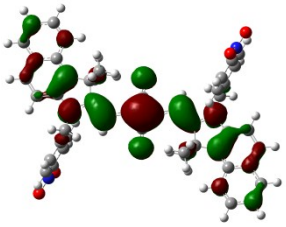
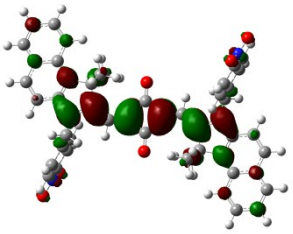
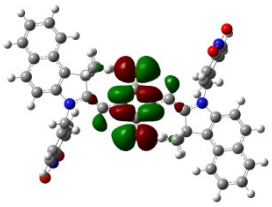
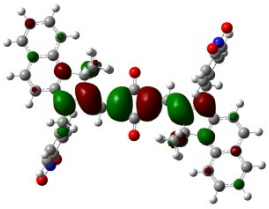
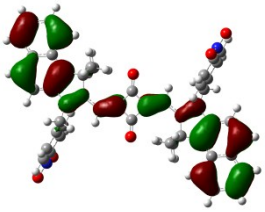
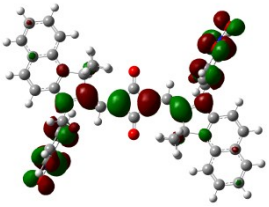
BIS-3	|1> w= 1.01082 f= 1.4499 2.1596 eV		
	|2> w= 0.99976 f= 0.0000 2.3556 eV		
	|3> w= 0.79338 f= 0.0000 2.8383 eV		

Fig.S1. Natural transition orbitals describing the first and second optimized ground states (absorption). The numbers in the left column indicate the corresponding excited-state number, fraction of the NTO pair contribution into the given electronic excitation, w; the excitation energy in eV; and f, the oscillator strength for the one-photon-absorption (OPA) excitations.

3. **Digital photo of BISs in solution**

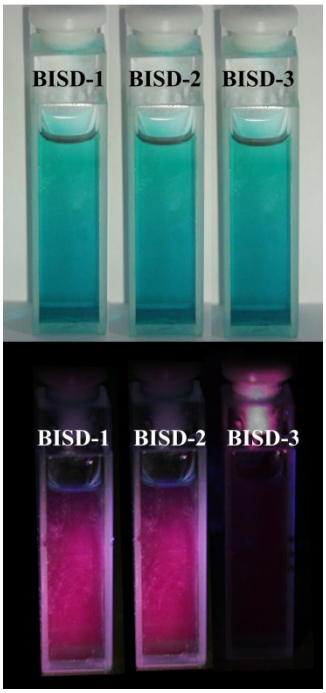
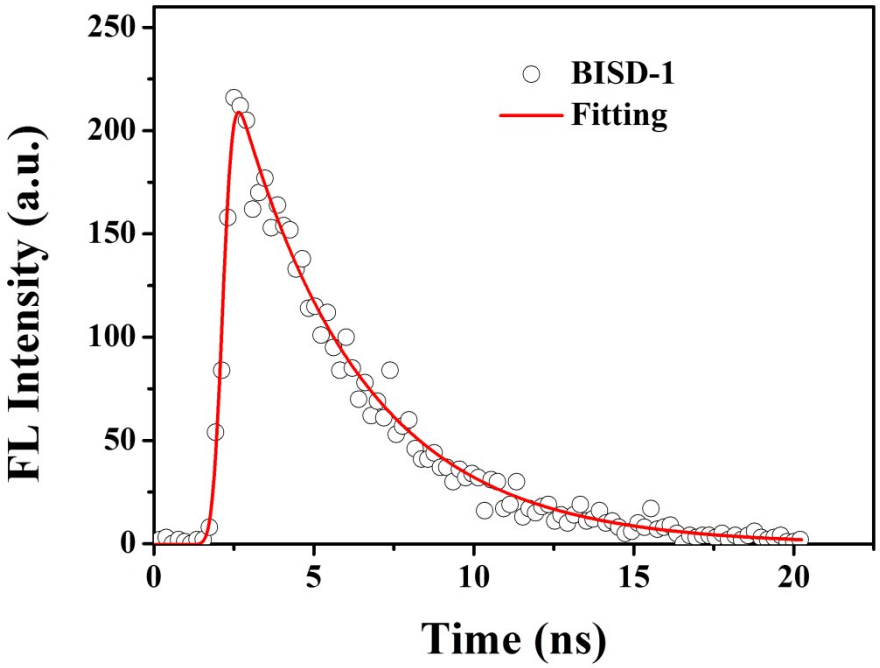
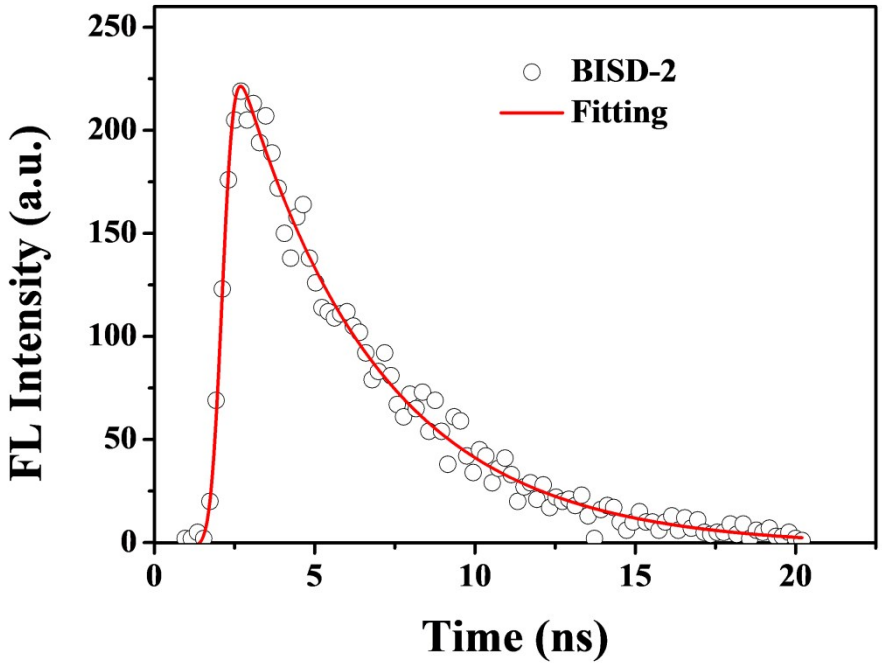


Fig.S2. Digital photo of BISs in THF (top) and under UV irradiation (bottom).

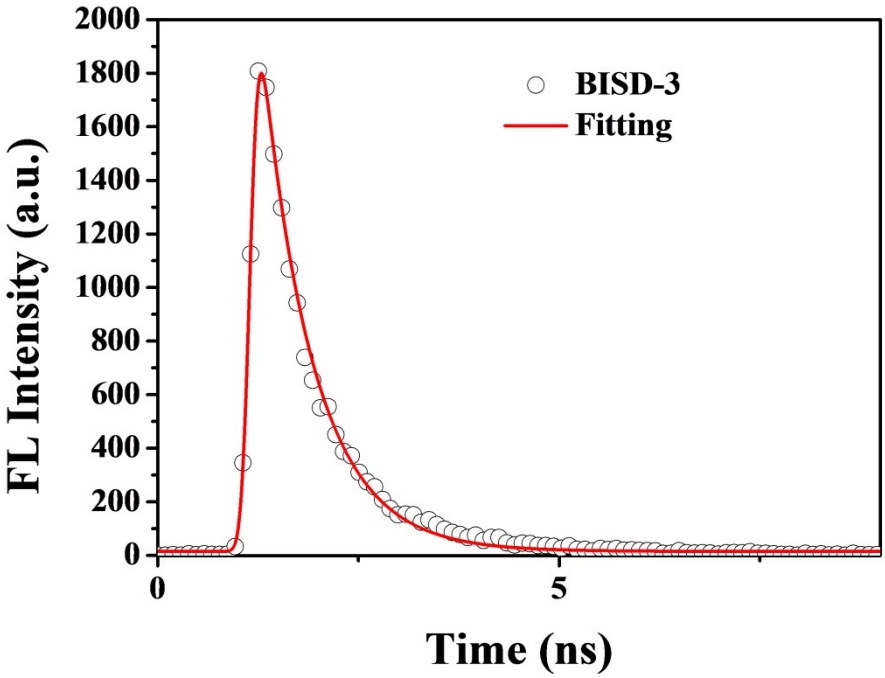
4. **The time-resolved fluorescence spectra**



BIS-1 τ=3.89 ns 0.64 R^2=0.98



BIS-2 τ=4.36 ns R^2=0.98



BIS-3 τ= 0.64 ns R^2=0.97

Fig.S3. The time-resolved fluorescence spectra of BISs in THF solutions. The scatter dot present the experiment fluorescence decay curves, and the black curves show the fitting line. The life of fluorescence (τ) and coefficient of determination (R^2) are provided with each charts.

5. Simulation of TPA cross section based on simplified sum-over-states model

From a simplified sum-over-states approach the cross-section for S_2 and S_3 will be approximated as[3, 4]:

$$\delta = K\frac{\mu_{gi}^2\mu_{if}^2}{(E_{gi} - E_{gf}/2)^2}$$

Table S1 the simplified SOS simulation result

	μ_{01}	μ_{12}	μ_{13}	E_{01}	E_{02}	E_{03}	δ	δ^*
BIS-1	5.45	0.030	4.70	2.15	2.41	2.80	0.030K	1166.5K
BIS-2	5.40	0.014	4.82	2.15	2.40	2.82	0.0063K	1237.1K
BIS-3	5.23	0.015	4.06	2.16	2.36	2.84	0.0063K	823.4K

μ and E present the transition moment and energy gap, the number show the related state. δ is the simulated TPA cross section based on the simplified SOS function, K is a constant. δ^* is the simulated TPA cross section based on the three states of S_0, S_1, S_3.

6. The cytotoxicity of the BIS-1 dye

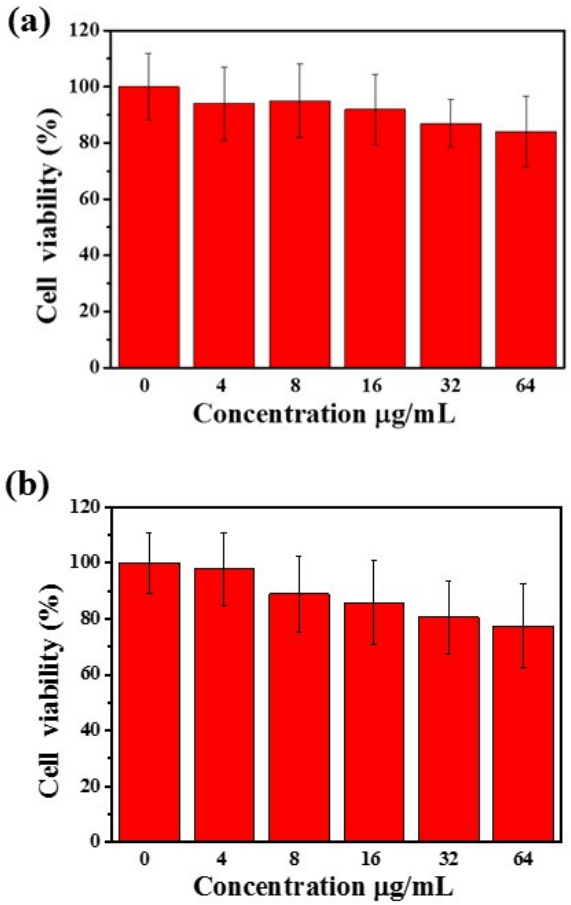


Fig. S4 The viability of MCF-7 (a) and fibroblast (b) cells at various concentrations of BIS-1 dye.

7. Steady-state one-photon absorption and fluorescence spectra of BIS-1 in DMSO/H_2O

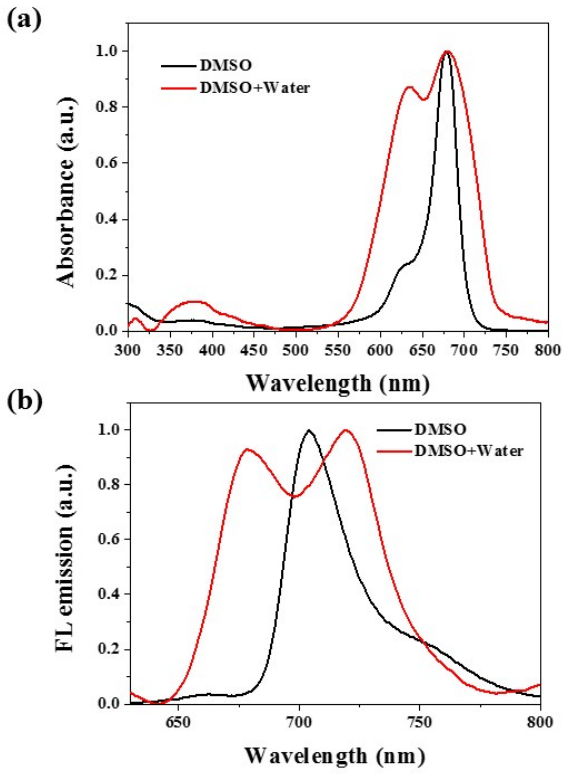


Fig. S5. Steady-state one-photon absorption and fluorescence spectra of BIS-1 in DMSO and DMSO/H_2O (V:V=1:9) solution

8. TPA spectrum of BIS-1 in DMSO/H_2O solution

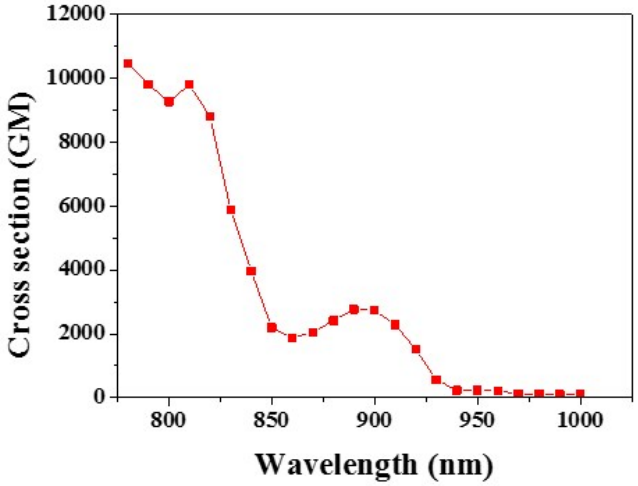


Fig. S6. TPA spectrum of BIS-1 in DMSO/H_2O (V:V=1:9) solution.

7. The two-photon laser confocal scanning microscopy images compared between Rhodamine B and BIS-1 stained cells.

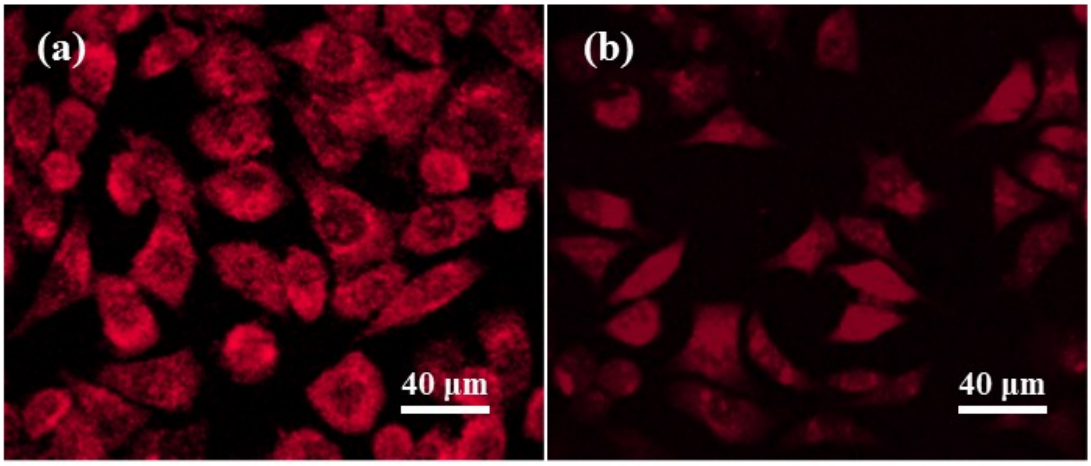


Fig. S7. Two-photon laser confocal scanning microscopy (TPLCSM) image of fibroblasts cells incubated with BIS-1 (a) and Rhodamine B (b) upon excitation at 800 nm

We have compared the TPLCSM images of the the mouse fibroblasts cells stained by both Rhodamine B and BIS-1(Fig. S7). With the same excitation energy and exposure time, the BIS-1 stained cells are only slightly brighter than that of Rhodamine B, which can be attributed to the lower concentration of BIS-1 due to poor water-solubility. The resolution of the BIS-1 stained cells are much higher owning to the much higher TPA cross section.

9. Reference

1. S. Kuster and T. Geiger, *Dyes Pigments*, 2012, **95**, 657-670.
2. N. Barbero, C. Magistris, J. Park, D. Saccone, P. Quagliotto, R. Buscaino, C. Medana, C. Barolo and G. Viscardi, *Org Lett*, 2015, **17**, 3306-3309.
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