Supplementary Information

An ESIPT Fluorescent Probe Sensitive to Protein α-helix Structures

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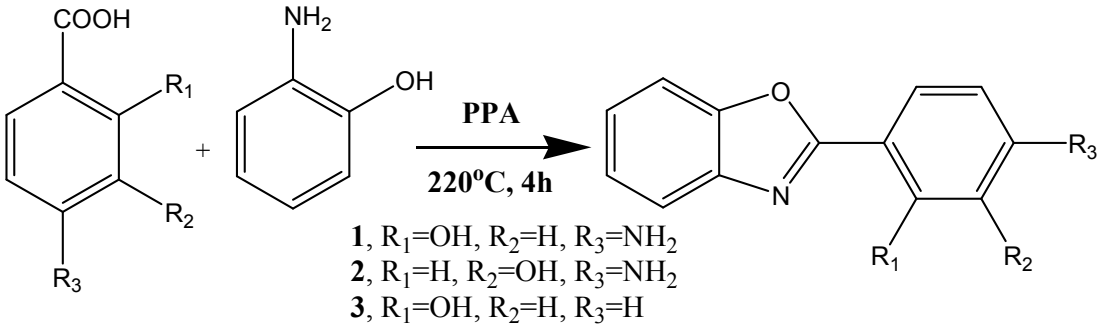


Fig. S1. The synthesis of **1**, **2**, **3**: 4-amino salicilic acid (10 mM) and 2-amino phenol (12 mM) were mixed in polyphosphoric acid (PPA) to synthesis **1** at 220 °C for 4 h. For **2**, the raw material was 4-amino-3-hydroxybenzoic acid, and for **3,** the starting was salicilic acid.

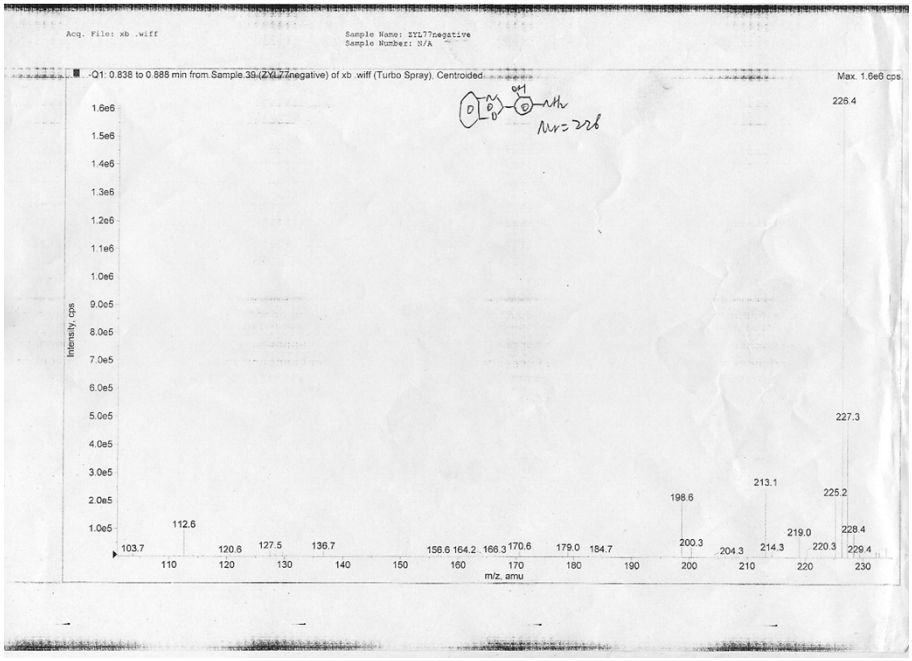
5-amino-2-benzooxazol-2-yl-phenol, 1: Yield, 80%. ESI-MS: m/z 226 (M^+); ^{1}H NMR (400 MHz, DMSO): δ 6.10 (s, 2H), 6.19 (d, J=2.0 Hz, 1H), 6.29 (dd, J_1= 2.0 Hz, J_2=8.8 Hz, 1H), 7.37–7.34 (m, 2H), 7.72–7.64 (m, 3H), 11.17 ppm (s, 1H); ^{13}C NMR (400 MHz, DMSO): δ 163.62, 159.86, 154.61, 148.23, 139.86, 128.32, 124.78, 124.26, 117.87, 110.33, 107.18, 99.29, 97.80ppm; elemental analysis calcd (%) for $C_{13}H_{10}N_2O_2$: C 69.07, H 4.46, N 12.39; found: C 69.25, H 4.23, N 12.38.

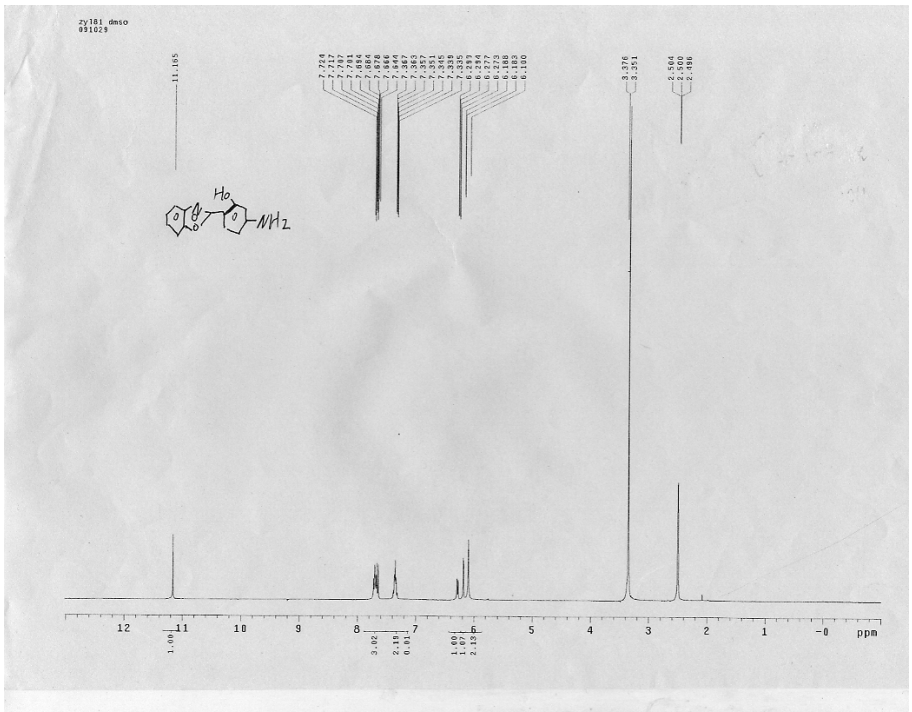
6-amino-3-benzooxazol-3-yl-phenol, 2: Yield, 76%. ESI-MS: m/z 226 (M^+); ^{1}H NMR (400 MHz, DMSO): δ 5.42 (s, 2H), 7.22 (d, J=7.6 Hz, 1H), 7.31 (t, J=3.6 Hz, 2H), 7.47 (d, J=9.6 Hz, 2H), 7.66 (s, 2H), 9.62 ppm (s, 1H); ^{13}C NMR (400 MHz, DMSO, ppm): δ 163.61, 149.90, 143.58, 142.11, 141.46, 124.30, 124.01, 120.31, 118.72, 113.40, 113.20, 112.54, 110.22; elemental analysis calcd (%) for $C_{13}H_{10}N_2O_2$: C 69.07, H 4.46, N 12.39; found: C 70.65, H 5.47, N 9.07.

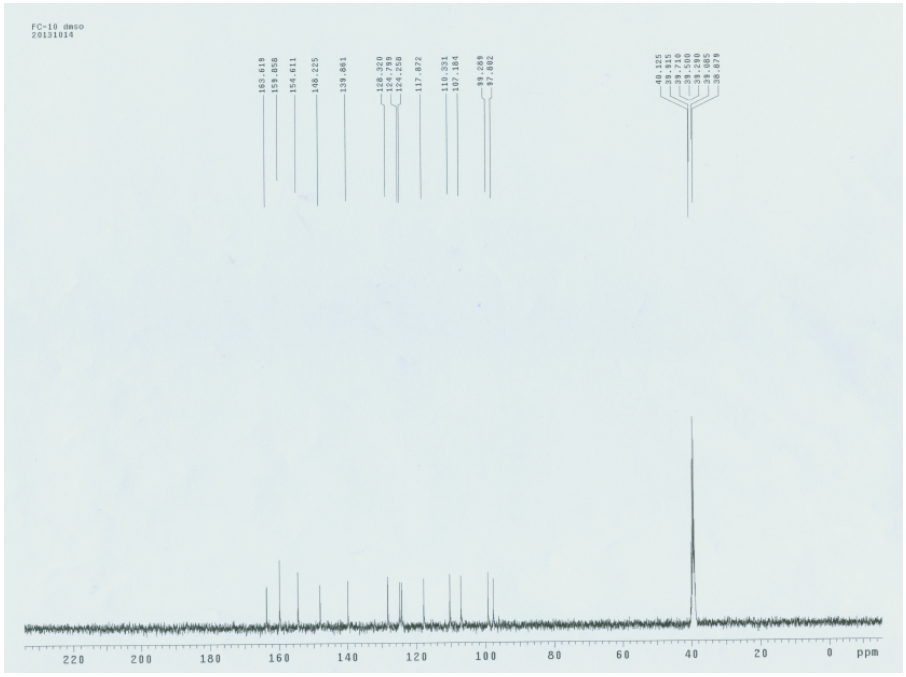
2-benzooxazol-2-yl-phenol, 3: Yield, 79%. ESI-MS: m/z 211(M^+); ^{1}H NMR (400 MHz, DMSO): δ 7.15–7.08 (m, 2H), 7.56–7.45 (m, 3H), 7.86 (t, J=2.8 Hz, 2H), 8.04 (d, J=7.6 Hz, 1H), 11.22 ppm (s, 1H); ^{13}C NMR (400 MHz, DMSO): δ 162.20, 157.71, 148.67,139.34, 133.83, 127.40, 125.74, 125.20, 119.84,

119.07, 117.08, 110.93, 110.23 ppm. elemental analysis calcd (%) for $C_{13}H_9NO_2$: C 73.98, H 4.30, N 6.64; found: C 73.10, H 4.67, N 6.59.

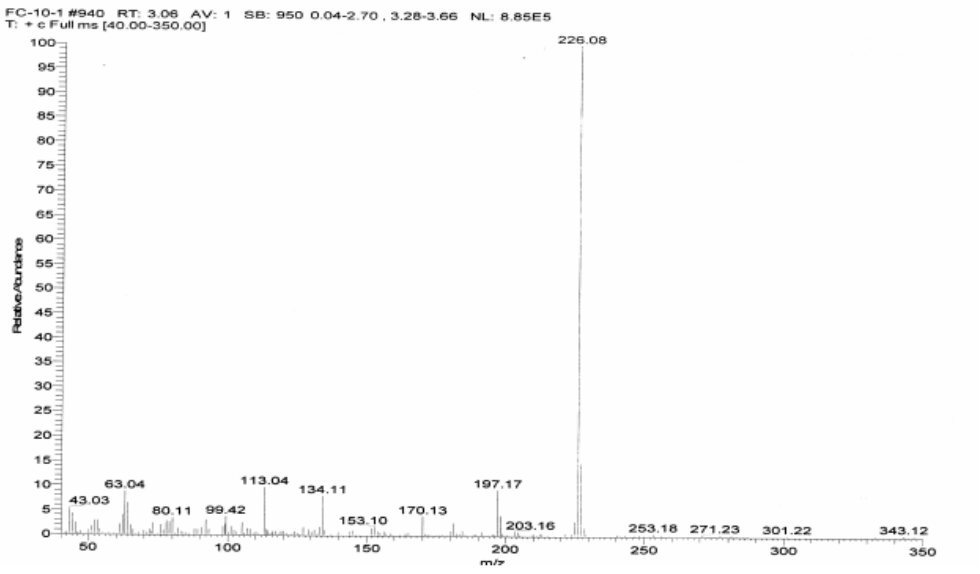
The MS, ^{1}H NMR and ^{13}C NMR of **1**.

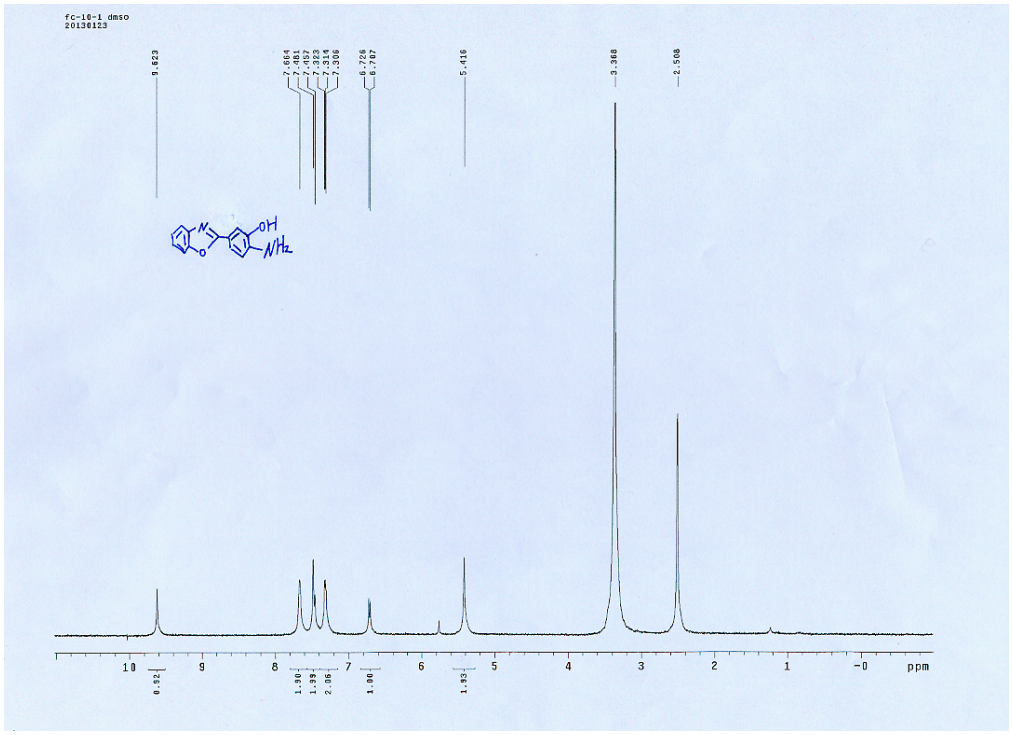


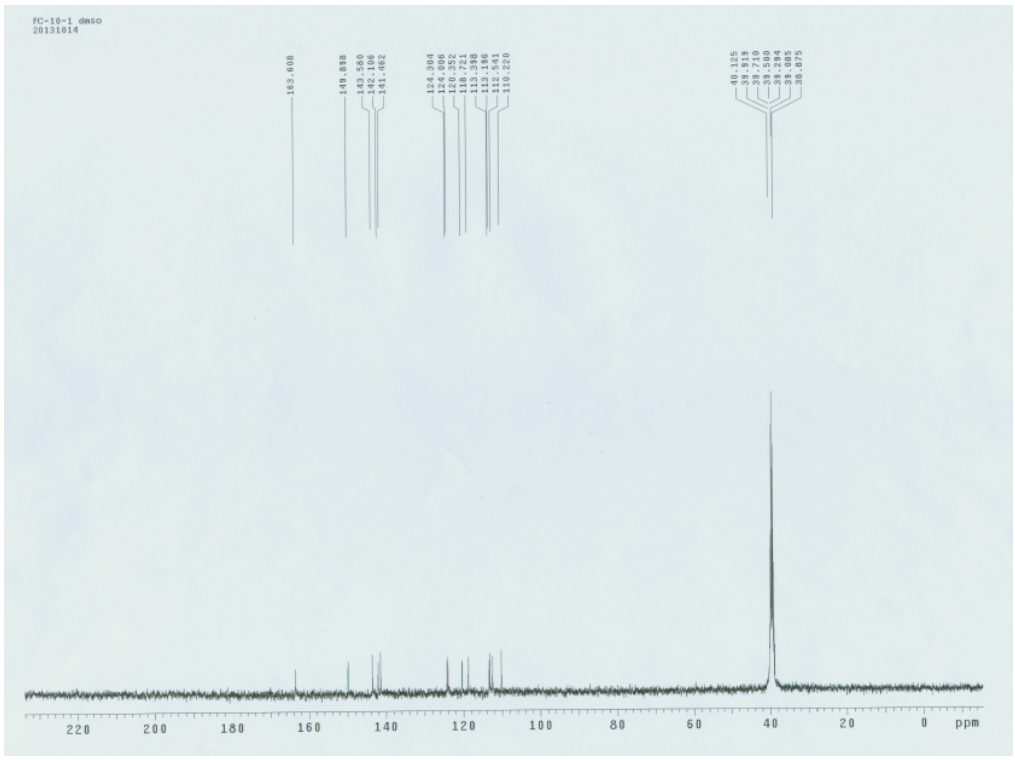




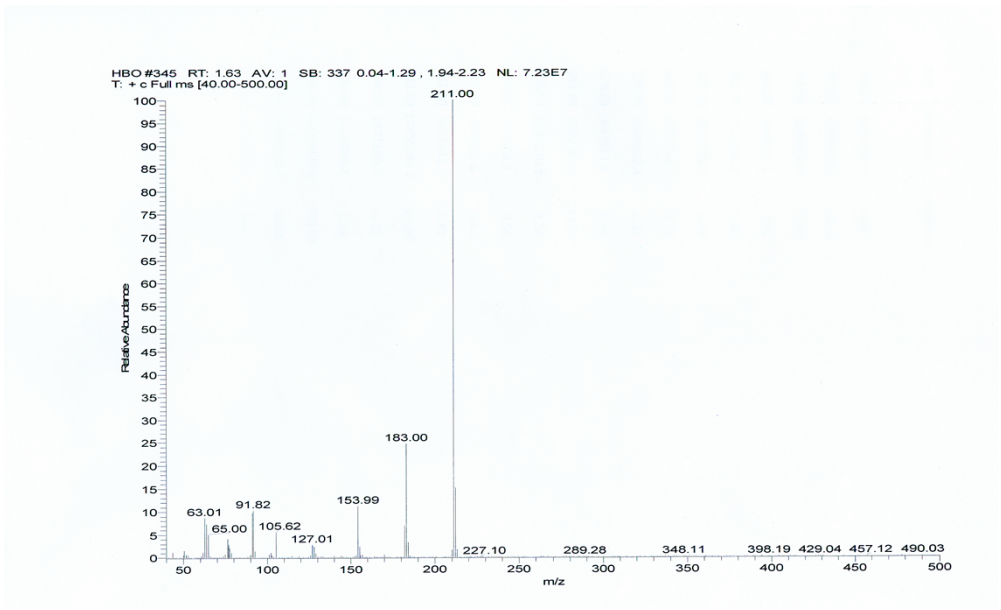
The MS, ^{1}H NMR and ^{13}C NMR of **2**.







The MS, ^{1}H NMR and ^{13}C NMR of **3**.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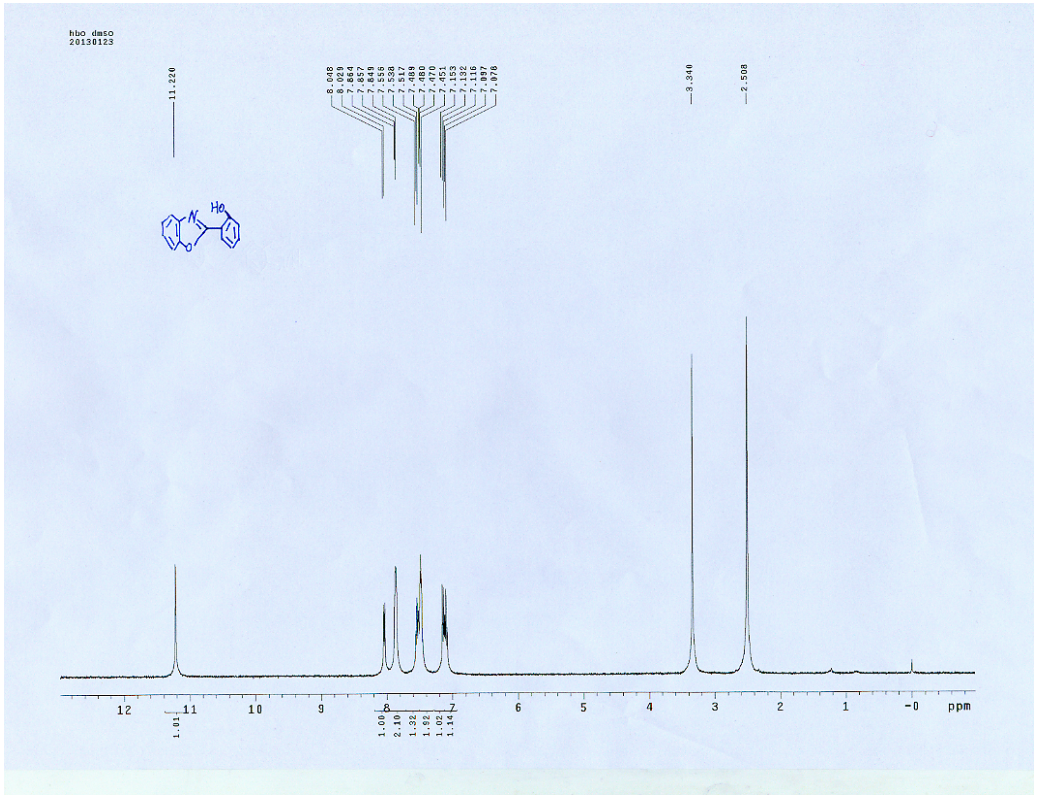
490.03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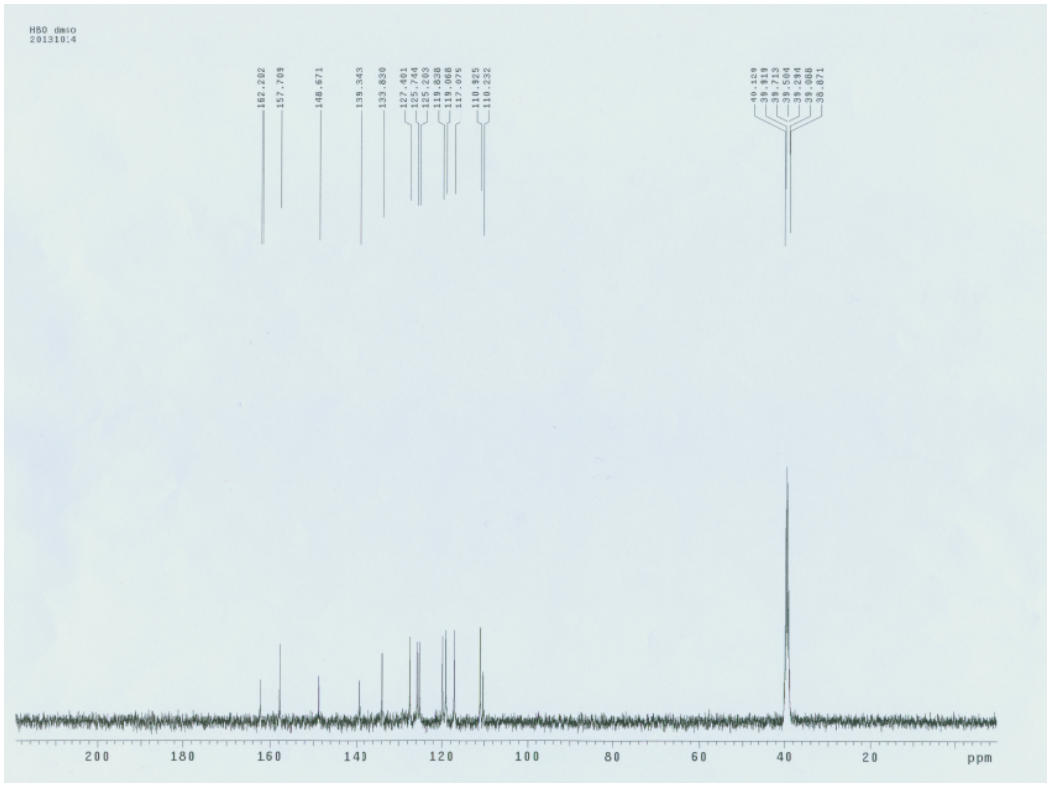

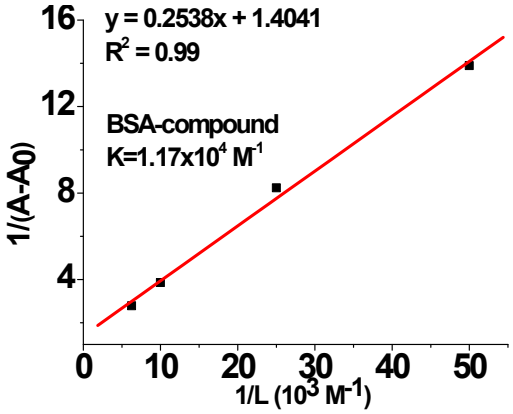

Fig. S2. The plot of 1/(A－A0) vs 1/L for BSA protein and **1** complexes where A_0 is the initial protein absorption band (270 nm) and A is the recorded absorption at different drug concentrations (L). All fluorescence experiments were performed in 100 mM Tris-HCl buffer (pH 7.4, 150 mM NaCl) at 25°C. The binding constant is 1.17×10^4 M^{-1} at 298 K following the method of Ref [1]

[1] M. Purcell, J. F. Neault, H. A. Tajmir-Riahi, *Biochimica et Biophysica Acta*, 2000, **1478**, 61.

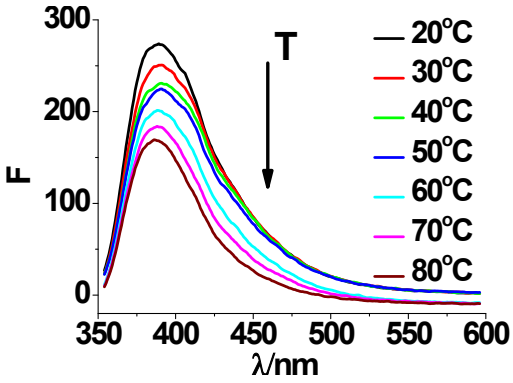

Fig. S3. Fluorescence spectra of 8 μM **1** at different temperature in 100 mM Tris-HCl buffer (pH 7.4, 150 mM NaCl). Arrow indicates the temperature change from 20 °C to 80 °C from up to bottom.

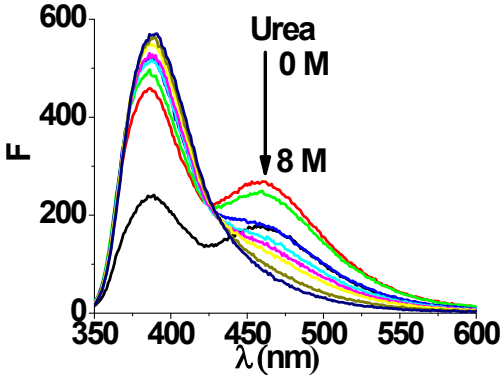

Fig. S4. Fluorescence responses of 8 μM **1** in 32 μM BSA exposed to 0–8 M urea. All experiments were performed in 100 mM Tris-HCl buffer (pH 7.4, 150 mM NaCl).

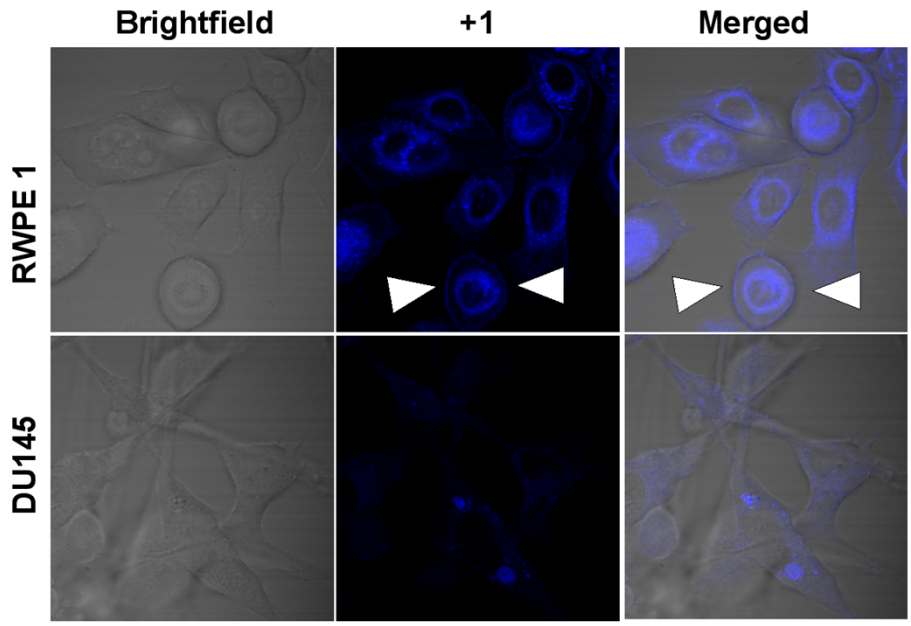

Fig. S5. Localization of **1**, respectively, in RWPE 1 and DU145 cells displayed by two-photon confocal microscopy. Cells were exposed to 4 μM **1** for 1 h prior to imaging. "▽" indicates cell membranes. × 63 (Plan-Apo, NA 1.4, oil immersion) objective was employed for imaging.