Supporting Information

A novel water-soluble fluorescent probe with ultra-sensitivity over a wider pH range and its application for differentiating cancer cells from normal cells

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1. Materials and instruments

Except for special labels, chemical reagents were obtained from commercial vendor (Shanghai Civic Chemical Technology Co., Ltd.) and employed without further purification. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument (the supplier from Shimadzu, Japan). ^{1}H and ^{13}C NMR data were obtained by Bruker AV-400 NMR spectrometer (the supplier from Shimadzu, Japan). Absorption spectra were obtained by UV-3101PC spectrophotometer (the supplier from Shimadzu, Japan). Fluorescence spectra were obtained by Horiba FluoroMax-4 spectrophotometer (the supplier from HORIBA Scientific, America). Fluorescence imaging of pH in live cells was carried out on an Olympus FV1000-IX81 confocal fluorescence microscope (the supplier from Olympus Corporation, Japan).

2. Calculation of the pK_a and K_a

According to the Henderson-Hasselbach-type mass action equation (log [(F_{max} − F)/ (F − F_{min})] = pK_a − pH, where F is the fluorescence emission intensity at 530 nm), we calculated that the pK_a of the probe is 8.976, and the K_a of probe **pH-DCN** is 1.06 × 10^{-9}.

3. Absorption spectra of different concentrations probe pH-DCN in aqueous solution

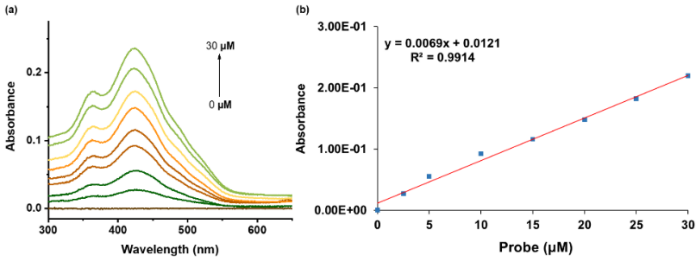


Figure S1 (a) The absorption spectra of different concentrations probe **pH-DCN** (0-30 μM) in the PBS solution (10 mM, pH = 4). (b) The absorbances of different concentrations probe **pH-DCN** as a function of probe concentrations (0-30 μM).

4. Determination of quantum yield

The $\Phi_{pH=4}$ (probe **pH-DCN** at pH value was 4) and $\Phi_{pH=12}$ (probe **pH-DCN** at pH value was 12) were determined in aqueous solution with optically matching solutions of fluorescein (Φ= 0.95 in 0.1 M NaOH solution)[1] as the standard and the quantum yield was calculated using the following equation:

$$\Phi_s = \Phi_r (A_r F_s / A_s F_r) (n_s^2 / n_r^2)^2$$

Where, s and r denote sample and reference, respectively. *A* is the absorbance. *F* is the relative integrated fluorescence intensity and n is the refractive index of the solvent, $\Phi_{pH=4}$ was calculated as 0.0018, $\Phi_{pH=12}$ was calculated as 0.236.

5. Cytotoxicity assays

The cell viability of RAW 264.7 macrophage cells, treated with probe **pH-DCN**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, RAW 264.7 macrophage cells, seeded at a density of 1×10^6 cells mL^{-1} on a 96-well plate, were maintained at 37 ℃ in a 5% CO_2 / 95% air incubator for 12 h. Then the live RAW 264.7 macrophage cells were incubated with various concentrations (0, 5, 10, 20, and 30 μM) of probe **pH-DCN** suspended in culture medium for 8 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

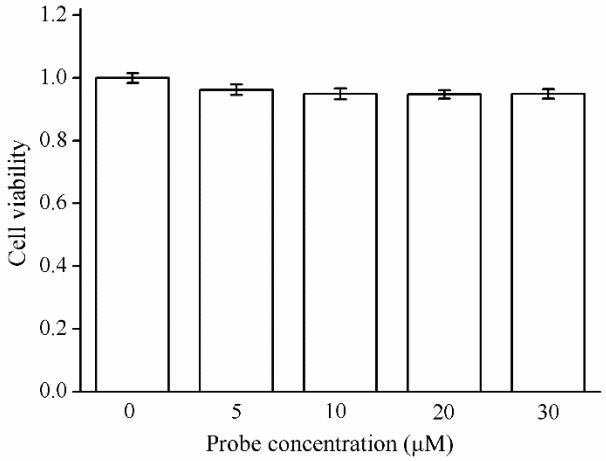


Figure S2 Cytotoxicity assays of probe **pH-DCN** at different concentrations for RAW 264.7 macrophage cells

6. Imaging studies of live cells

The RAW 264.7, HEK293, HUVEC, MGC-803, A549, HeLa and PC12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO_2 at 37 ℃ humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin–streptomycin. Cells were seeded on dish for fluorescence microscopic imaging by laser confocal fluorescence microscope. The cells were incubated with the probe **pH-DCN** (10 μM) for 30 minutes, the cells were rinsed three times with potassium rich PBS at different pH values of 6.0 and 7.4. The cells were incubated further with nigericin (10 μM) for 30 minutes, and after the confocal cell imaging was conducted. Then the fluorescence imaging of cells was carried out by confocal fluorescence microscope. And HEK293, HUVEC, MGC-803, A549, HeLa and PC12 cells were incubated with probe **pH-DCN** (10 μM) for 60 min, finally the fluorescence imaging of cells was carried out by confocal fluorescence microscope.

7. The ^{1}H-NMR and ^{13}C-NMR data of probe pH-DCN

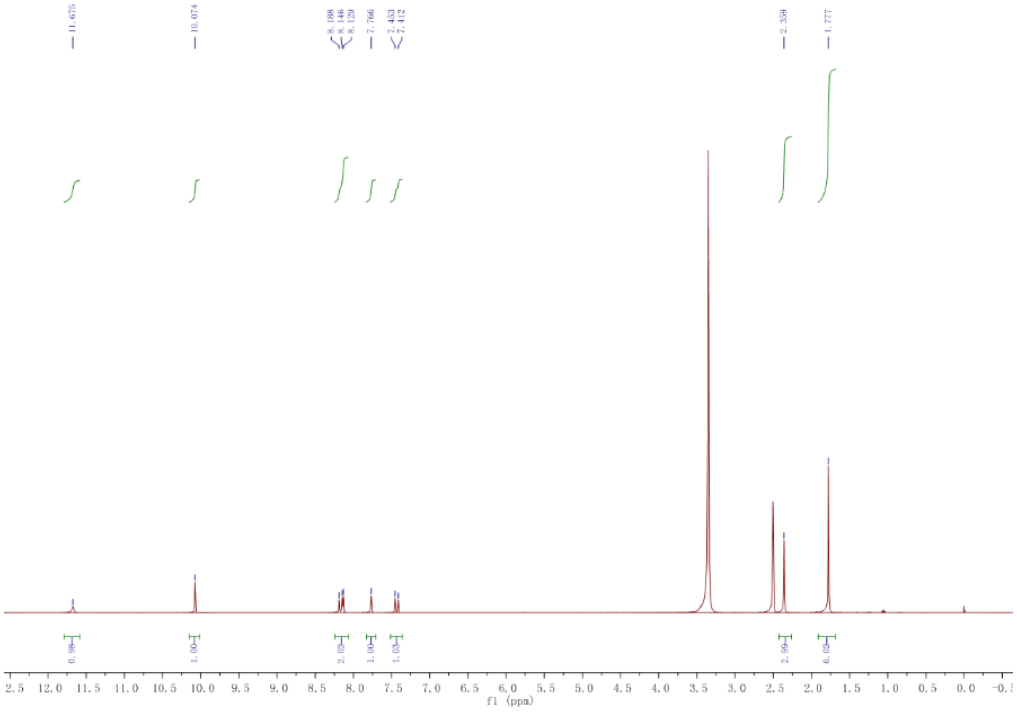


Figure S3 ^{1}H-NMR data of probe **pH-DCN**

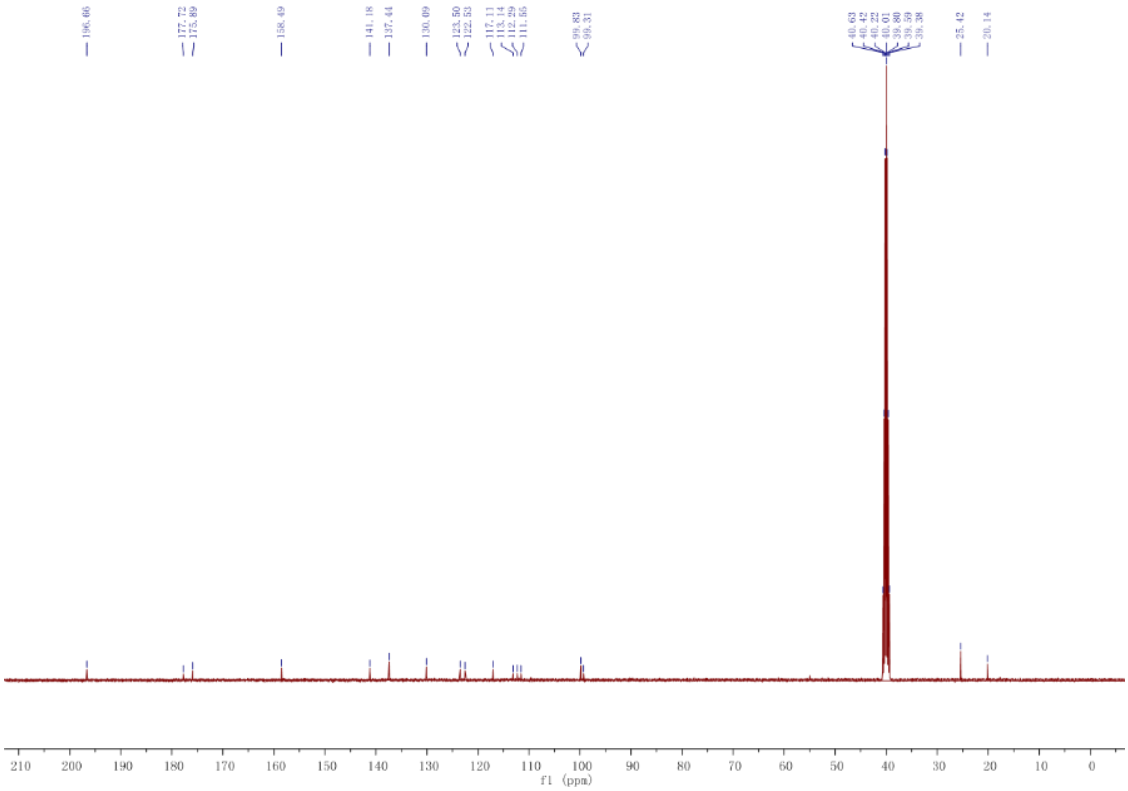
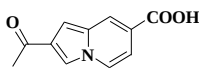
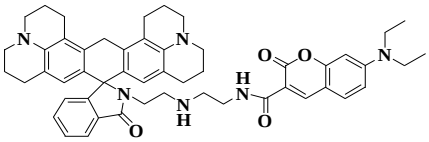
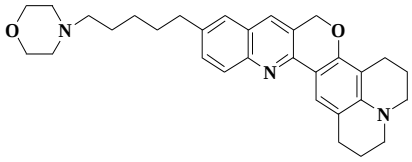
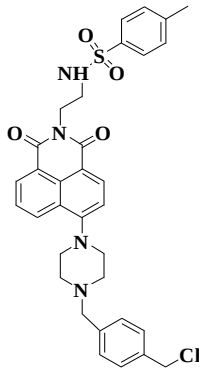
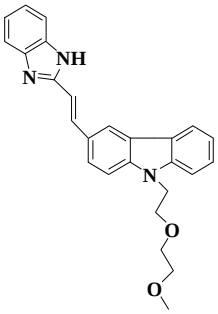
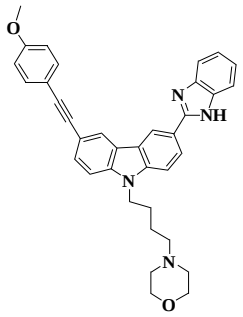
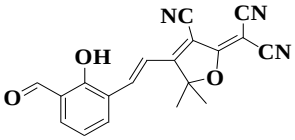


Figure S4 ^{13}C-NMR data of probe **pH-DCN**

8. Additional table of comparison between reported pH probes and pH-DCN

Table S1 Comparison of fluorescent probes for pH

Probe	λem	Solvent (v: v)	pH range	Distinguish cancer cells	Ref
	458	DMSO/H_2O (2: 8)	2.4–8.0	no	Sensors and Actuators: B 247 (2017) 46–52
	475/605	EtOH/H_2O (4: 6)	4.5–8.5	no	ACS Sens. 2 (2017) 436–442
	560/613	CH_3CN/H_2O (3: 7)	2–9	no	Anal. Chem. 89 (2017) 7038−7045
	528	DMSO/H_2O (1: 9)	2.5–.9.5	no	Sens. Actuators B 273 (2018) 1754–1761
	454/514	DMSO/H_2O (1: 4)	2.5–6.8	no	Sens. Actuators B 262 (2018) 913–921

	410/475	DMSO/H_2O (3: 7)	3.2–9.6	no	Chem. Commun. 55 (2019) 1782–1785
	530	Aqueous solution	4–12	yes	This work

9. References

1 T.-B. Ren, W. Xu, Q. -L. Zhang, X. -X. Zhang, S. -Y. Wen, H. -B. Yi, L. Yuan and X.-B. Zhang, *Angew. Chem. Int. Ed.*, 2018, **57**, 7473-7477.