

Electronic Supplementary Information (ESI) for

A Ratiometric Fluorescent pH Probe Based on Keto-Enol Tautomerization for Imaging of Living Cells in Extreme Acidity

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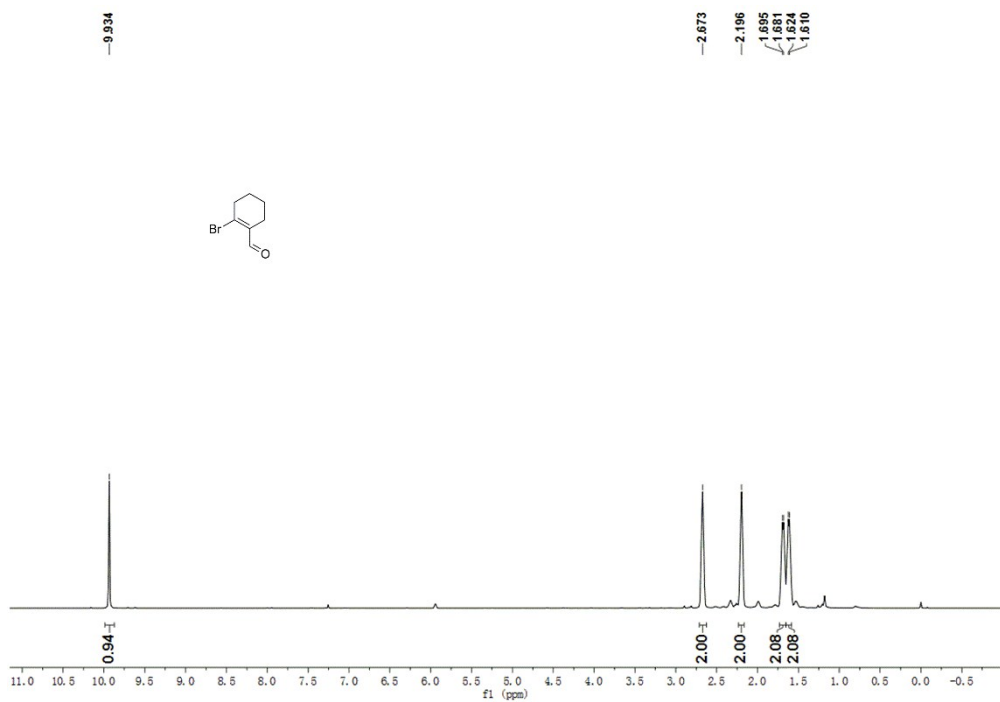


Figure S1. ¹H NMR spectrum of compound 1 in CDCl₃

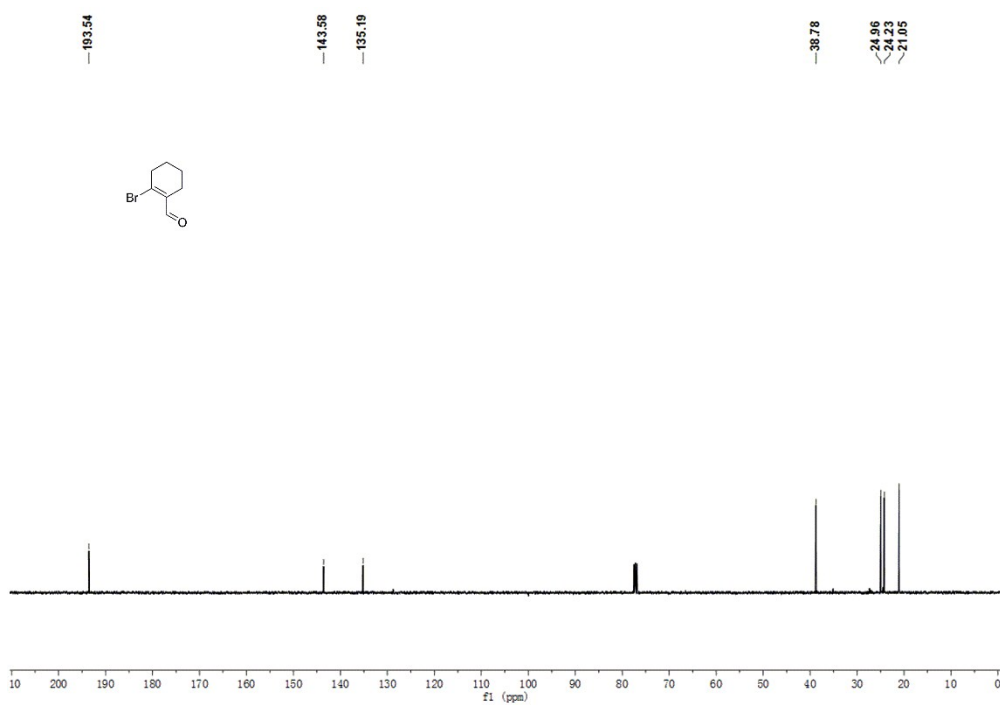


Figure S2. ¹³C NMR spectrum of compound 1 in CDCl₃

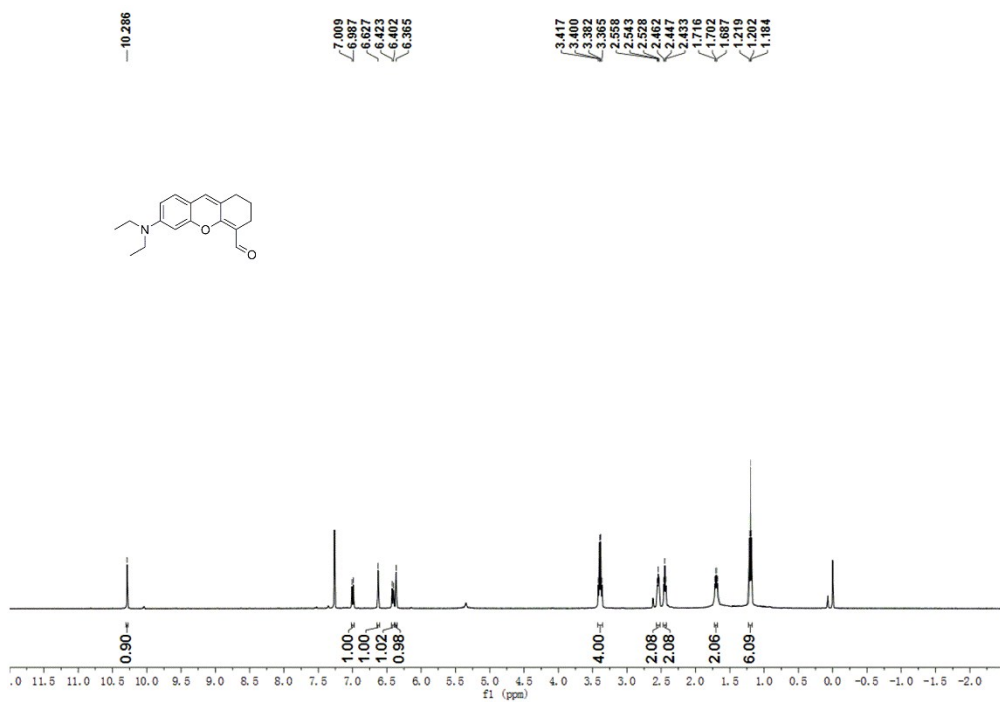


Figure S3. ¹H NMR spectrum of probe DDXC in CDCl₃

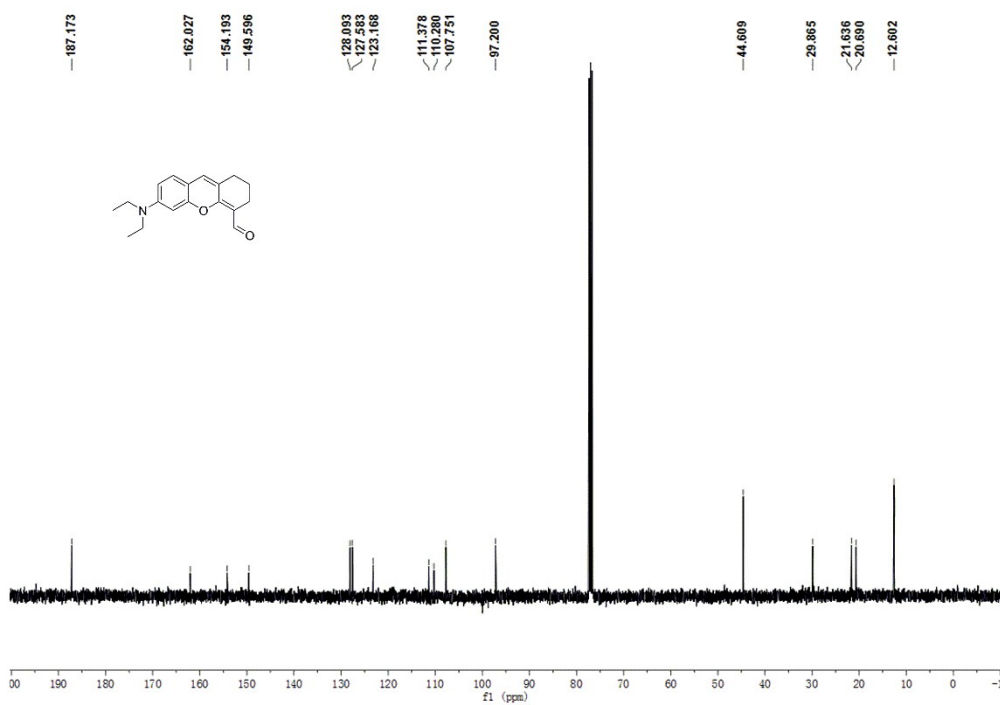
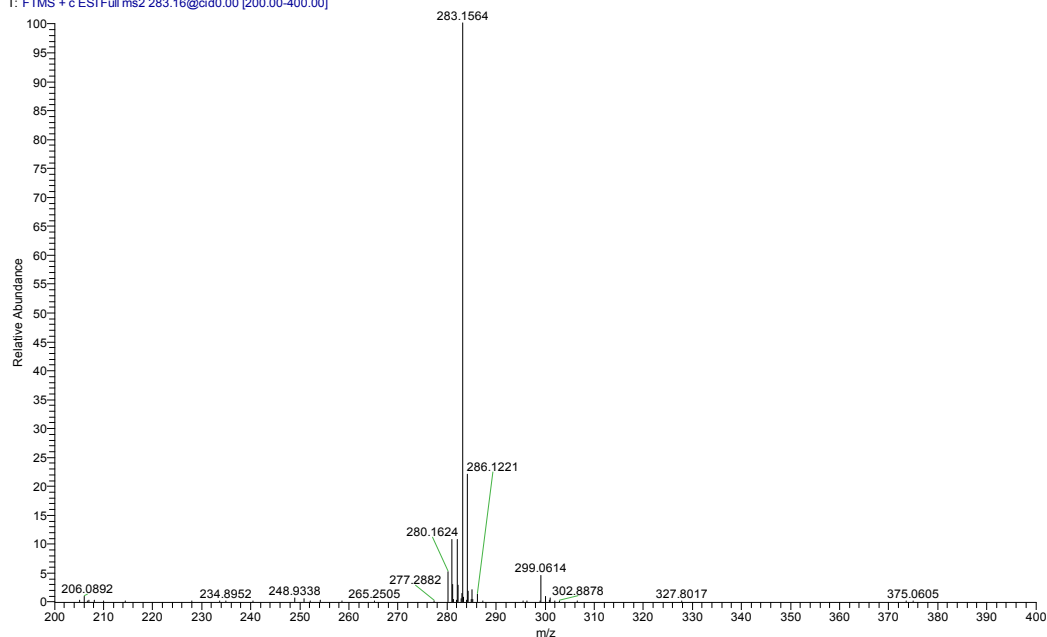


Figure S4. ¹³C NMR spectrum of probe DDXC in CDCl₃

TZX20170426 #11 RT: 0.09 AV: 1 NL: 1.58E6
 T: FTMS + c ESI Full ms2 283.16@cid0.00 [200.00-400.00]



File : C:\Xcalibur\data\hh-170426-283-hr-av2.RAW
 Full ms [260.500 - 317.500] - Range: 260.500 - 317.500
 Scan No. 1 of 1

Mass	Relative Intensity	Theoretical Mass	Delta [ppm]	Delta [mmu]	Composition
283.156	100.0	283.1567	-1.4	-0.4	C ₁₈ H ₂₁ O ₂ N ₁
		283.1441	43.0	12.2	C ₁₇ H ₁₉ O ₂ N ₂
		283.1693	-45.8	-13.0	C ₁₉ H ₂₃ O ₂
		283.1329	82.7	23.4	C ₁₈ H ₁₉ O ₃
		283.1805	-85.5	-24.2	C ₁₈ H ₂₃ O ₁ N ₂

Figure S5. HRMS of probe DDXC

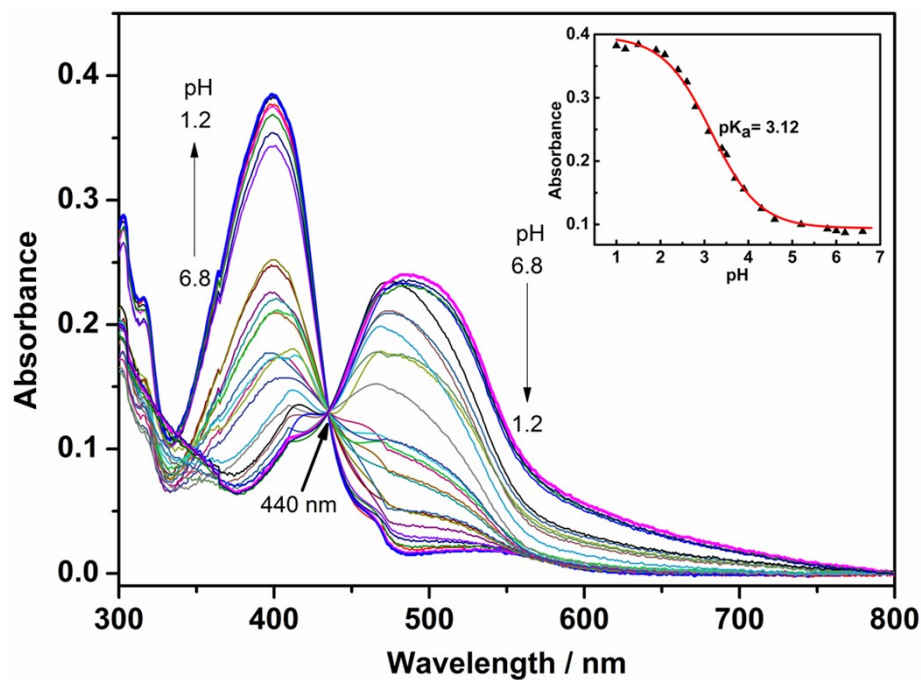
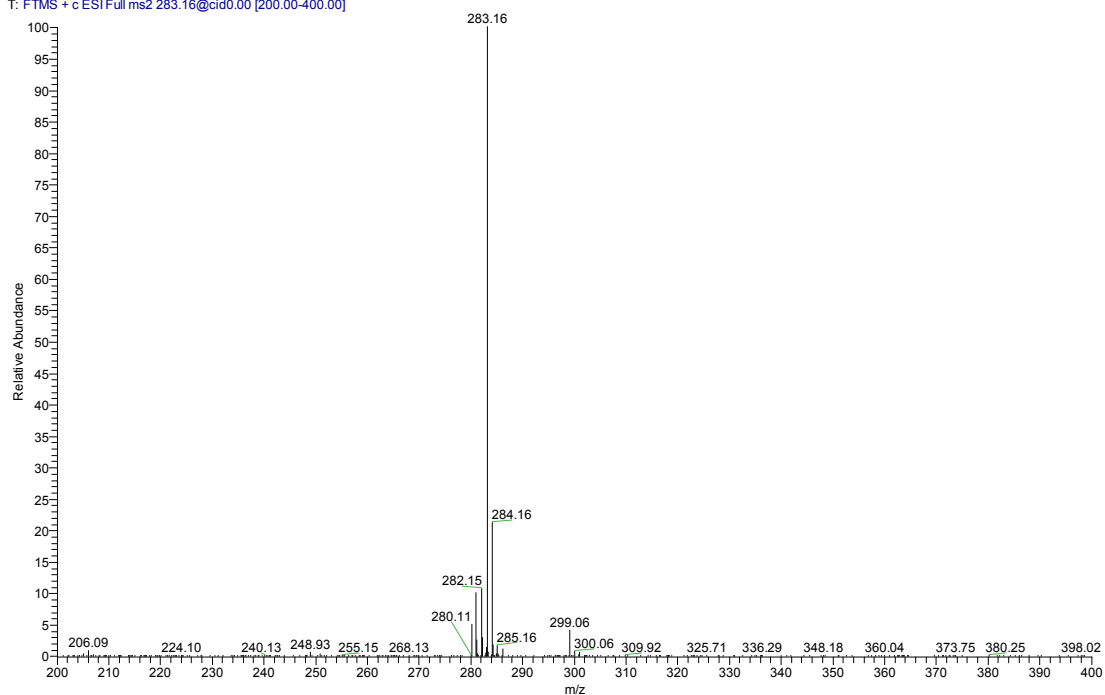


Figure S6. UV-vis absorption spectra of probe DDXC (30 μ M) recorded at different pH values from 6.8 to 1.2. Inset: Sigmoidal fitting of pH-dependent absorbance at 400 nm

a)

TZX20170426 #2-18 RT: 0.02-0.15 AV: 17 NL: 1.60E6
T: FTMS + c ESI Full ms2 283.16@cid0.00 [200.00-400.00]



b)

TZX20170426 #197 RT: 1.57 AV: 1 NL: 1.63E6
T: FTMS + c ESI SIM ms [280.66-285.66]

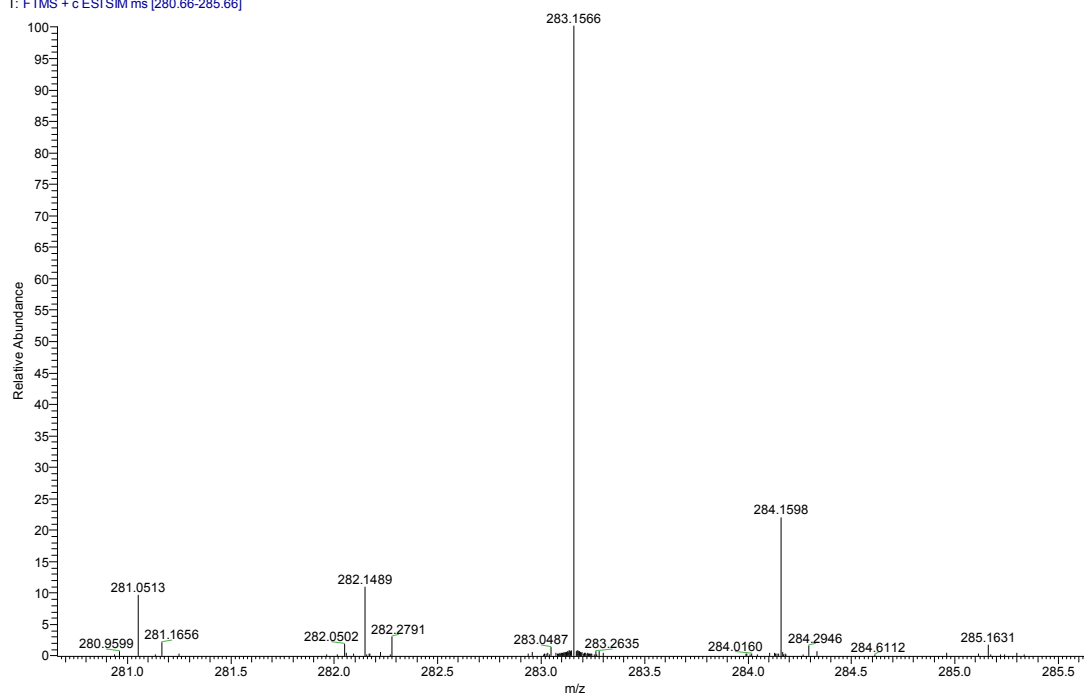


Figure S7. a) HRMS of probe DDXC (1.0 μ M) toward CF_3COOH ($\text{CF}_3\text{COOH}/\text{MeOH} = 1.0\%$, v/v); b) the partial enlarged drawing of a).

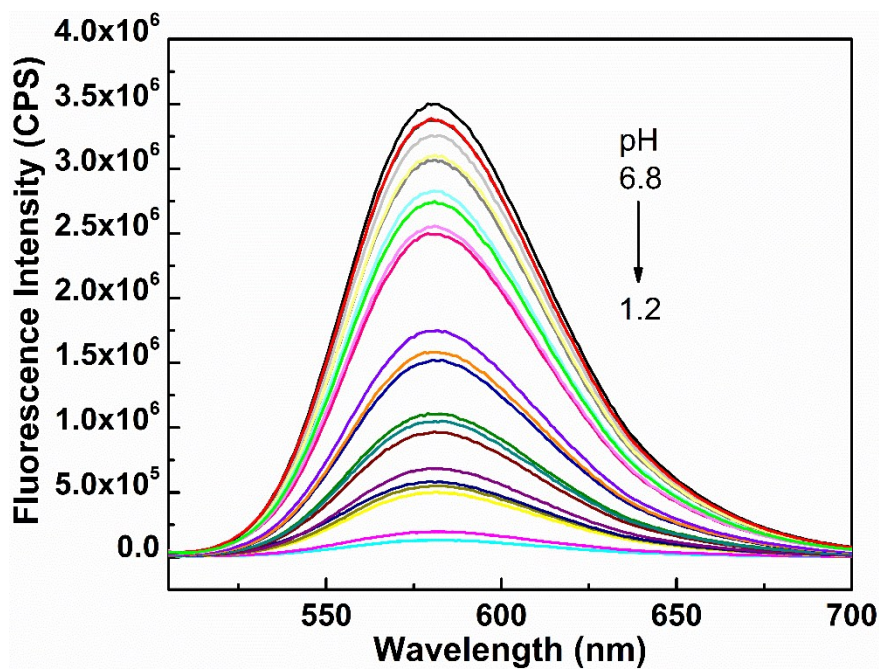


Figure S8. Fluorescence emission spectra of DDXC (10 μ M) in phosphate-citrate buffer (10 mM Na_2HPO_4 and 10 mM citric acid) as the pH value decreased from 6.8 to 1.2. $\lambda_{\text{ex}} = 490$ nm, excitation and emission bandwidths were both set at 10 nm.

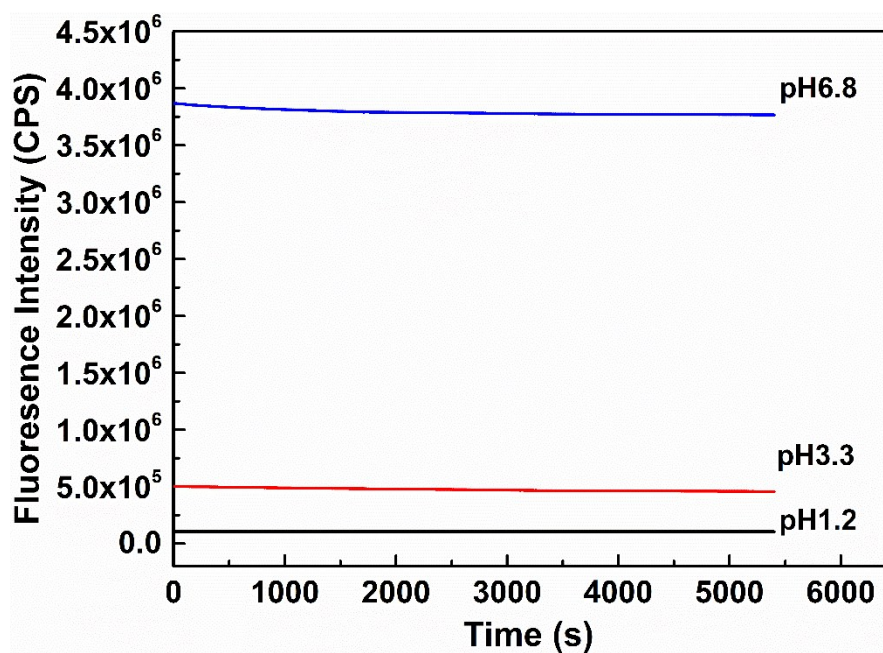


Figure S9. The time courses of fluorescence intensity of DDXC (10 μ M) in buffer pH 6.8, 3.3 and 1.2, respectively, $\lambda_{\text{ex/em}} = 490/580$ nm.

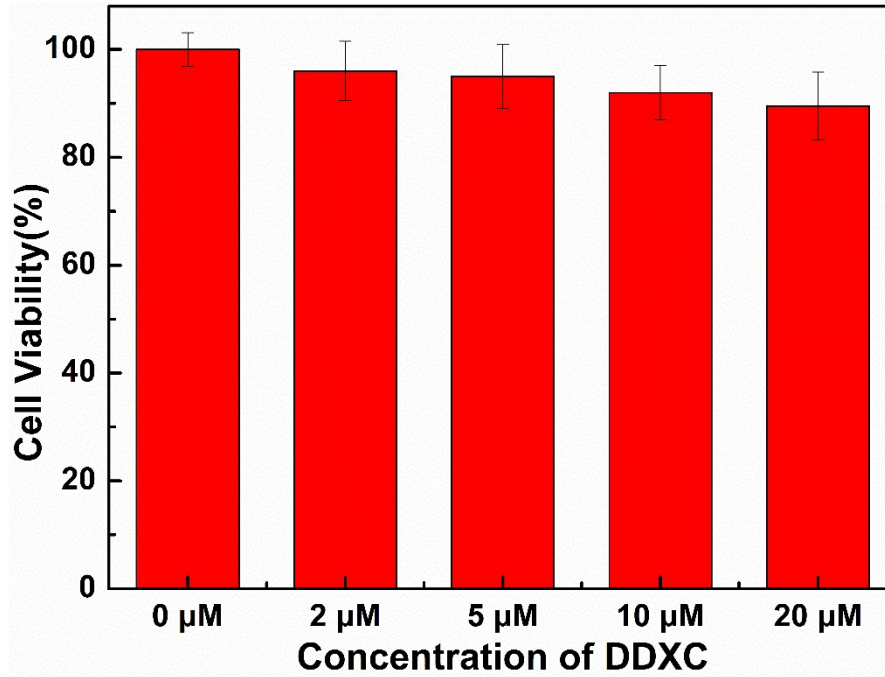


Figure S10. Cell cytotoxicity assay of probe DDXC: the cell viability values (%) are determined by incubated HeLa cells with DDXC of varying concentrations (0, 2, 5, 10 and 20 μM) for 12 h.