

## Fluorescent probes based on acridine derivatives and their application in dynamic monitoring of cell polarity variation

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**Table S1.** Optical properties of probe **1a** in different solvents.

Probe	Solvents	$\Delta f$	$\lambda_{\text{Abs,max}}^{\text{a}}$	$\lambda_{\text{Em,max}}^{\text{a}}$	Stokes shift <sup>a</sup>	$\epsilon^{\text{b}}$	$\Phi^{\text{c}}$
<b>1a</b>	H <sub>2</sub> O	0.3200	355	594	239	2.47	0.50
<b>1a</b>	DMSO	0.2640	351	583	232	3.34	3.06
<b>1a</b>	MeCN	0.3040	345	580	235	3.26	3.43
<b>1a</b>	MeOH	0.3092	345	574	229	3.88	3.83
<b>1a</b>	EtOH	0.2887	345	570	225	4.46	5.24
<b>1a</b>	DCM	0.2170	346	560	214	3.85	5.57
<b>1a</b>	THF	0.2086	347	562	215	3.57	5.82
<b>1a</b>	EA	0.1990	346	560	214	3.39	8.00
<b>1a</b>	Dioxane	0.0205	348	565	217	3.60	11.1
<b>1a</b>	TOL	0.0153	348	553	205	3.68	35.6

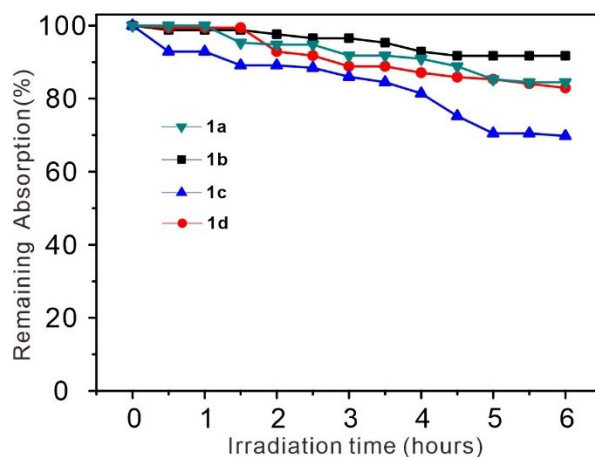
**Table S2.** Optical properties of probe **1b** in different solvents.

Probe	Solvents	$\Delta f$	$\lambda_{\text{Abs,max}}^{\text{a}}$	$\lambda_{\text{Em,max}}^{\text{a}}$	Stokes shift <sup>a</sup>	$\epsilon^{\text{b}}$	$\Phi^{\text{c}}$
<b>1b</b>	H <sub>2</sub> O	0.3200	364	572 <sup>d</sup>	208	1.00	0.51
<b>1b</b>	DMSO	0.2640	366	568 <sup>d</sup>	202	1.02	0.63
<b>1b</b>	MeCN	0.3040	366	557	191	0.98	1.42
<b>1b</b>	MeOH	0.3092	366	555	189	0.96	1.32
<b>1b</b>	EtOH	0.2887	366	556	190	0.87	1.47
<b>1b</b>	DCM	0.2170	367	548	181	1.04	2.18
<b>1b</b>	THF	0.2086	366	541	175	1.12	3.58
<b>1b</b>	EA	0.1990	364	535	171	1.06	9.93
<b>1b</b>	Dioxane	0.0205	364	535	171	1.24	13.5
<b>1b</b>	TOL	0.0153	366	534	168	0.97	13.2

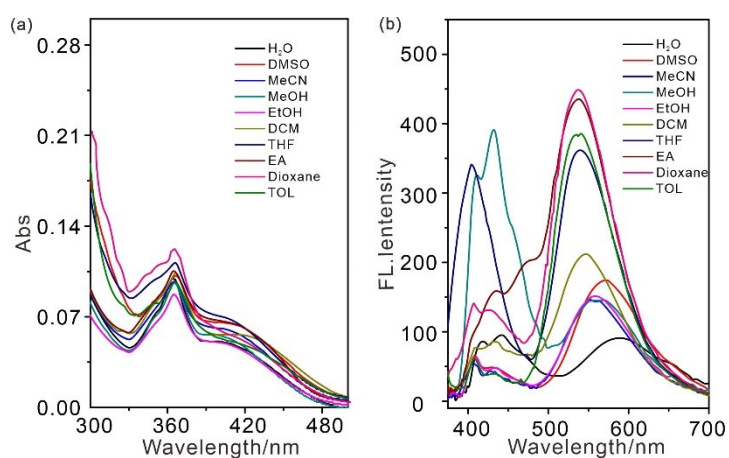
**Table S3.** Optical properties of probes **1c-d** in H<sub>2</sub>O and glycerol.

Probe	Solvents	$\lambda_{\text{Abs,max}}^{\text{a}}$	$\lambda_{\text{Em,max}}^{\text{a}}$	Stokes shift <sup>a</sup>	$\epsilon^{\text{b}}$	$\Phi^{\text{c}}$
<b>1c</b>	H <sub>2</sub> O	366	442	76	1.65	0.30
<b>1c</b>	Glycerol	362	445	83	0.93	12.48
<b>1d</b>	H <sub>2</sub> O	362	538	176	0.70	0.12
<b>1d</b>	Glycerol	365	532	167	0.98	9.20

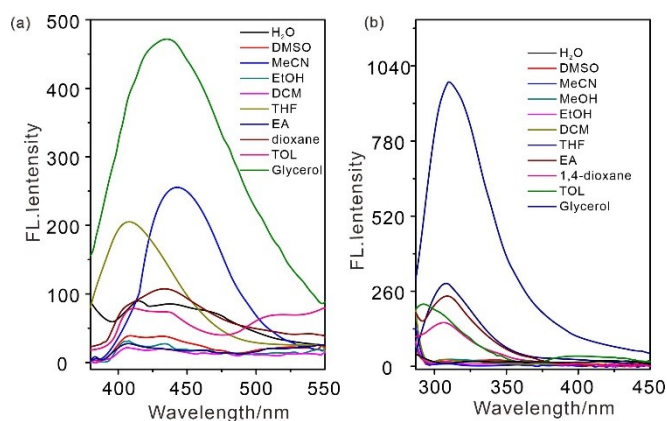
<sup>a</sup> Reported in nm.<sup>b</sup> Reported in 10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup>.<sup>c</sup> Reported in %. Coumarin-153 ( $\Phi=0.544$  in ethanol) was used as the reference compound in test.<sup>d</sup> Second highest peak.



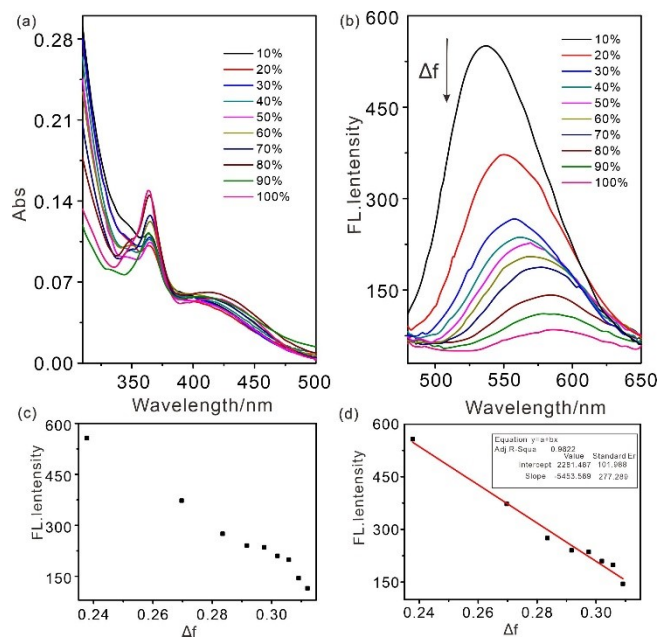
**Fig. S1.** Photofading behaviors of probes **1a-d** in acetonitrile.



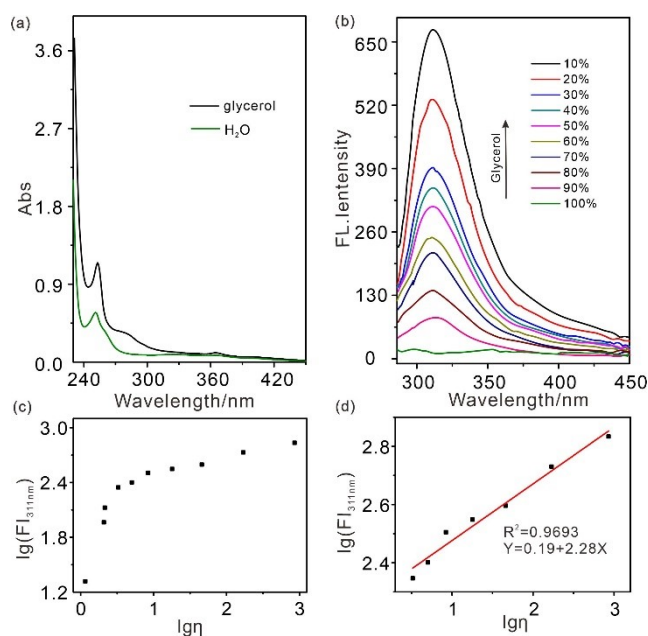
**Fig. S2.** Optical properties of probe **1b** (10 μM) in different solvents. (a) Absorption spectra; (b) emission spectra (excited at 362 nm, slit widths: 5 nm/10 nm).



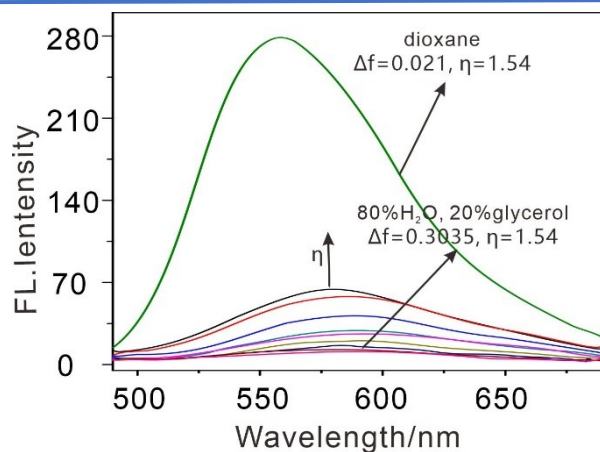
**Fig. S3.** Emission spectra of probes **1c-d** in different solvents. (a) emission spectra of **1c**; (b) emission spectra of **1d**.



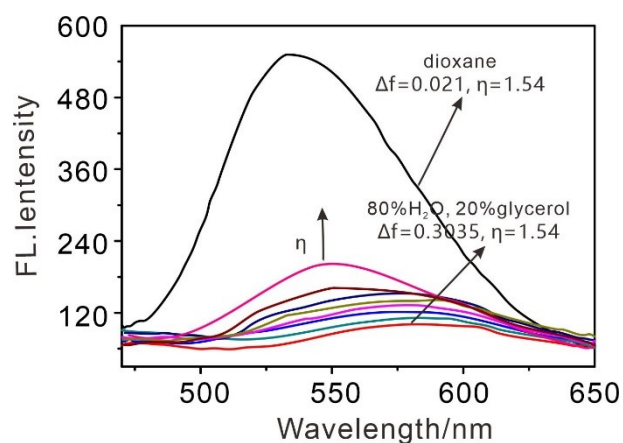
**Fig. S4.** Optical responses of probe **1b** (10  $\mu\text{M}$ ) in dioxane/ $\text{H}_2\text{O}$  mixtures with increasing polarity (water from 10% to 100%). (a) Absorption spectra; (b) emission spectra ( $\lambda_{\text{ex}}=362$  nm, slit widths: 5 nm/10 nm); (c) relationship between fluorescence intensity (570 nm) and  $\Delta f$ ; (d) linear relationship of fluorescence intensity at 570 nm versus  $\Delta f$  (0.238–0.315).



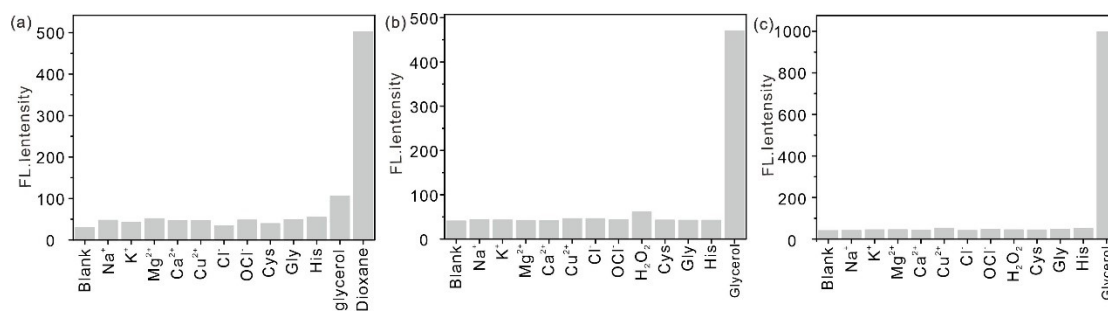
**Fig. S5.** Optical responses of probe **1d** (10  $\mu\text{M}$ ) in glycerol/ $\text{H}_2\text{O}$  mixtures with increasing viscosity (water from 100% to 1%). (a) Absorption spectra; (b) emission spectra ( $\lambda_{\text{ex}}=282$  nm, slit widths: 5 nm/10 nm); (c) relationship between  $\lg(I_{311\text{nm}})$  and  $\lg\eta$ ; (d) linear relationship of  $\lg(I_{311\text{nm}})$  versus  $\lg\eta$ .



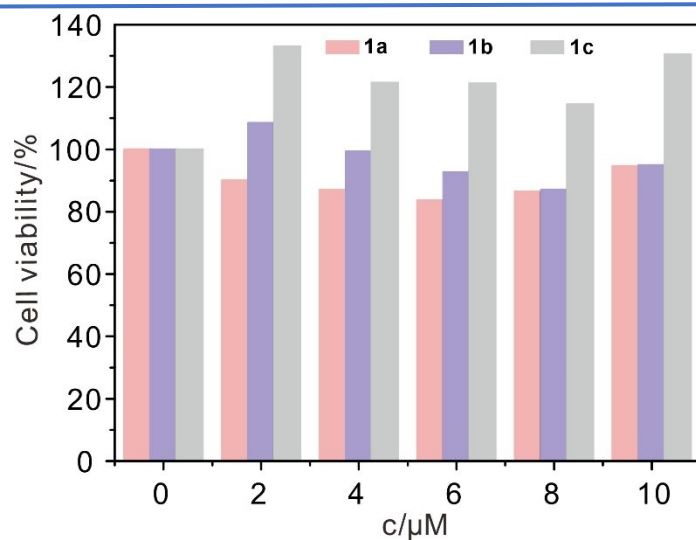
**Fig. S6.** The fluorescence spectra of probe **1a** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ /glycerol mixture under different viscosity.



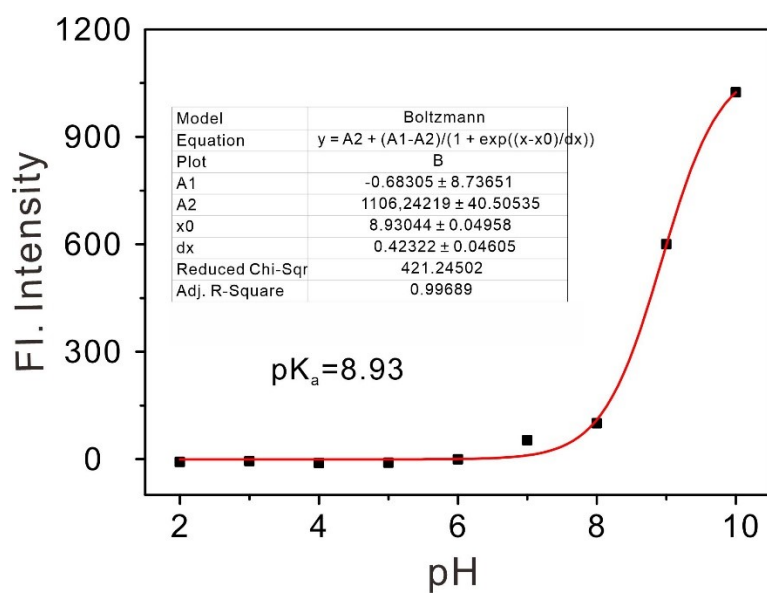
**Fig. S7.** The fluorescence spectra of probe **1b** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ /glycerol mixture under different viscosity.



**Fig. S8.** Selectivity experiments of probes **1b-d** (10  $\mu\text{M}$ ) toward different analytes. 5 mM for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{OCl}^-$ ,  $\text{H}_2\text{O}_2$ ; 10 mM for  $\text{K}^+$ ,  $\text{Na}^+$ , Cys, Gly, His. (a) probe **1b** ( $\lambda_{\text{ex}}=362$  nm, slit widths: 5 nm/10 nm). (b) probe **1c** ( $\lambda_{\text{ex}}=360$  nm, slit widths: 5 nm/10 nm). (c) probe **1d** ( $\lambda_{\text{ex}}=282$  nm, slit widths: 5 nm/10 nm).

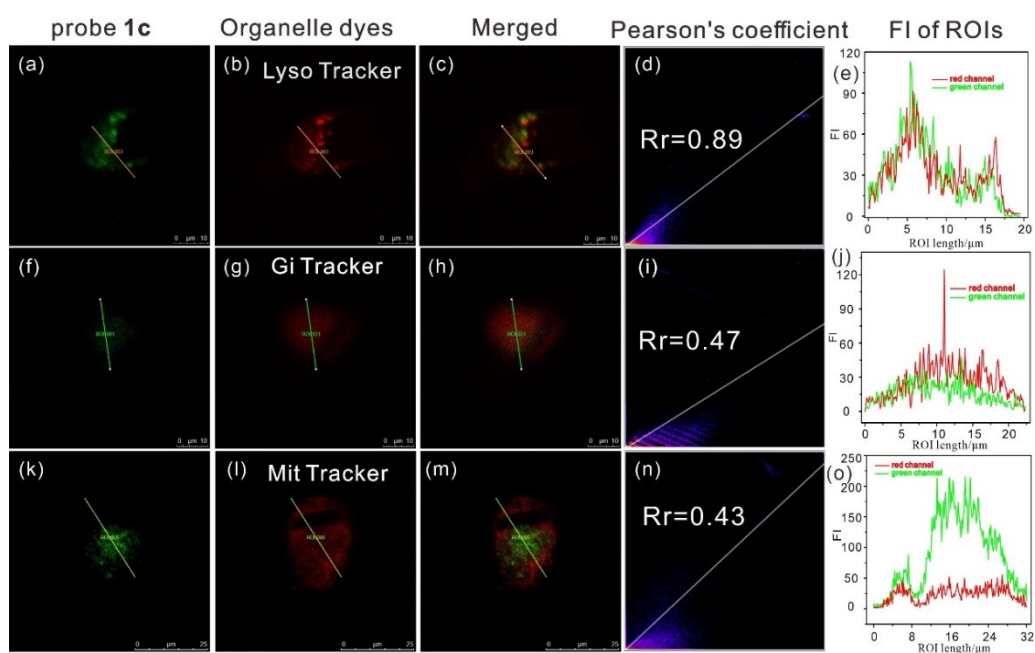


**Fig. S9.** HeLa cells viabilities after treatment with probes **1a-c**. Cell viability was assayed by the CCK-8 method.

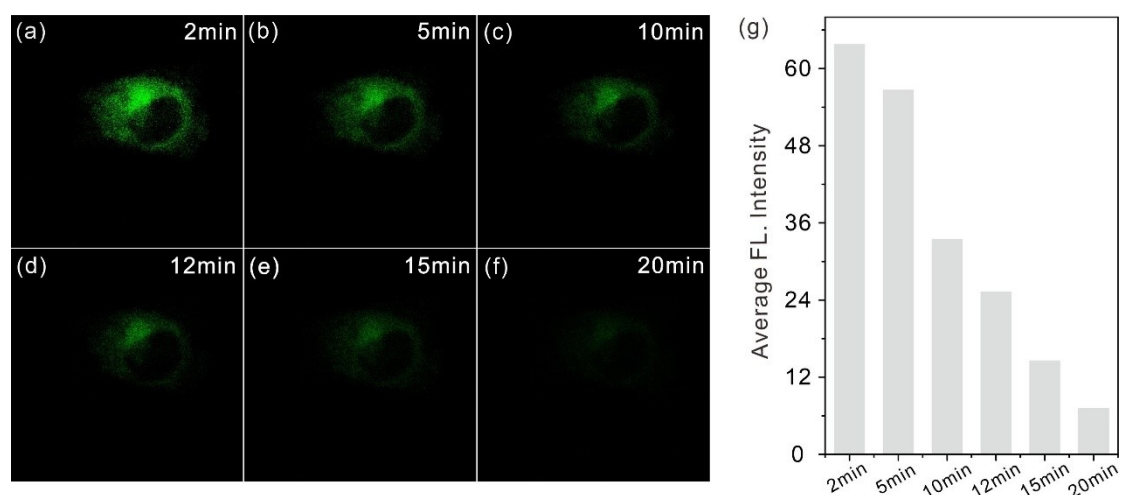


**Fig. S10.** Fluorescence intensity of probe **1b** at different pH.

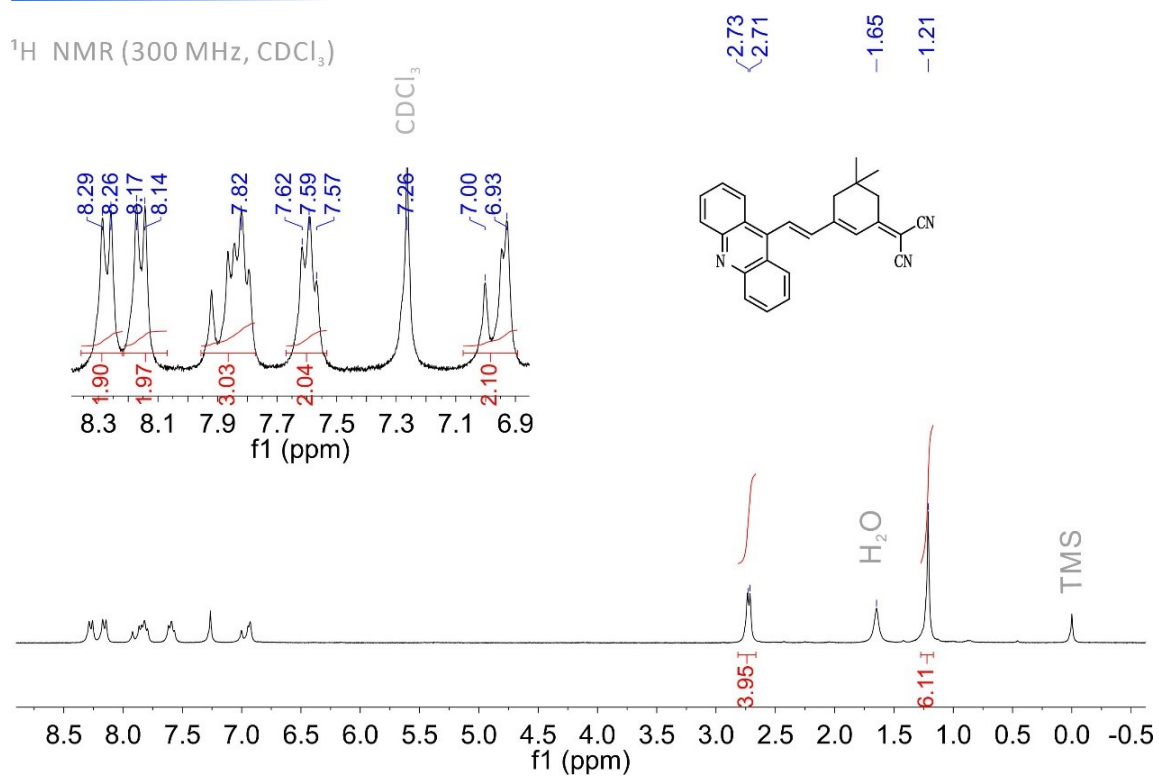
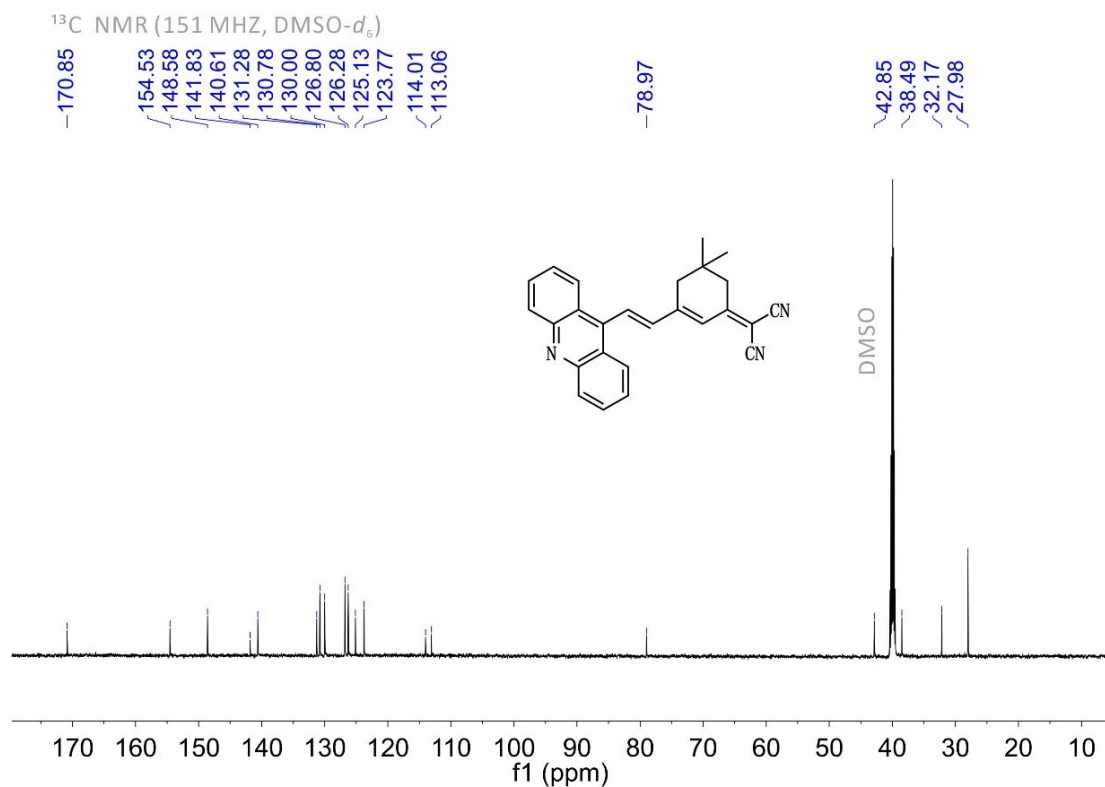


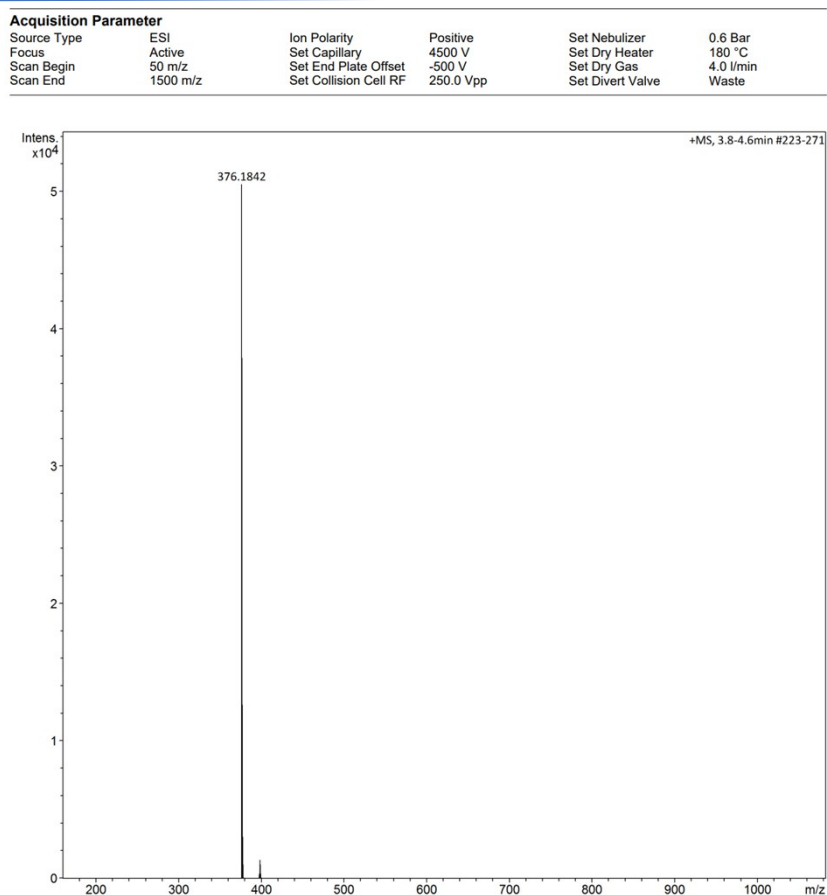
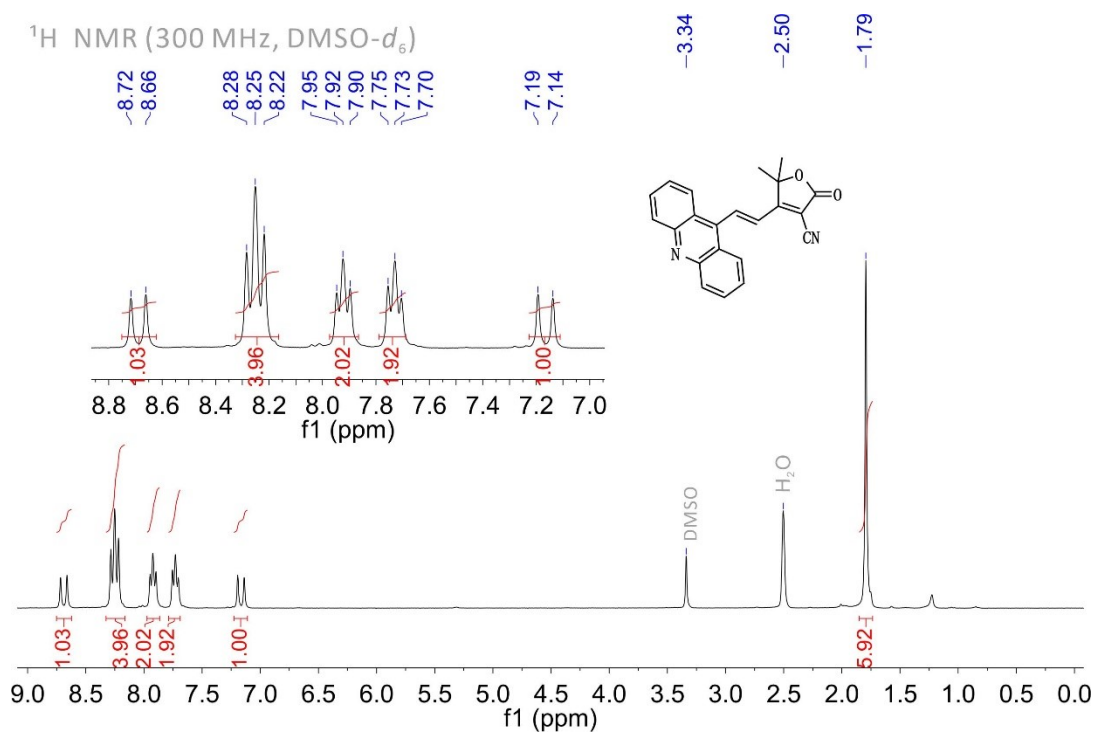


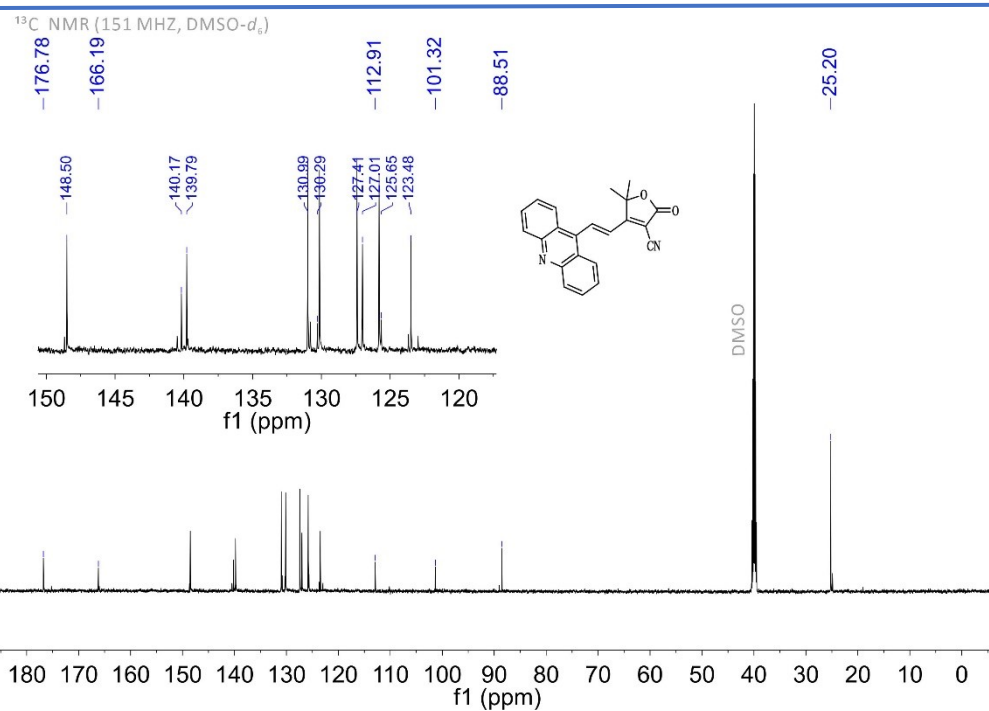
**Fig. S11.** Fluorescence confocal images of living HeLa cells with probe **1c** and ROI analysis: (a, f, k) confocal image (green channel) of cells with probe **1c** (6  $\mu\text{M}$ ); (b, g, l) confocal image (red channel) of cells with Lyso-Tracker Red DND-99 (100 nM), Gi-Tracker Red (100 nM) or Mito-Tracker<sup>®</sup>Red CMXRos (100 nM); (c, h, m) merged image of the green and red channels; (d, i, n) fluorescence intensity correlation plot of the green and red channels; (e, j, o) fluorescence intensities of the regions of interest (ROIs) across the cells.



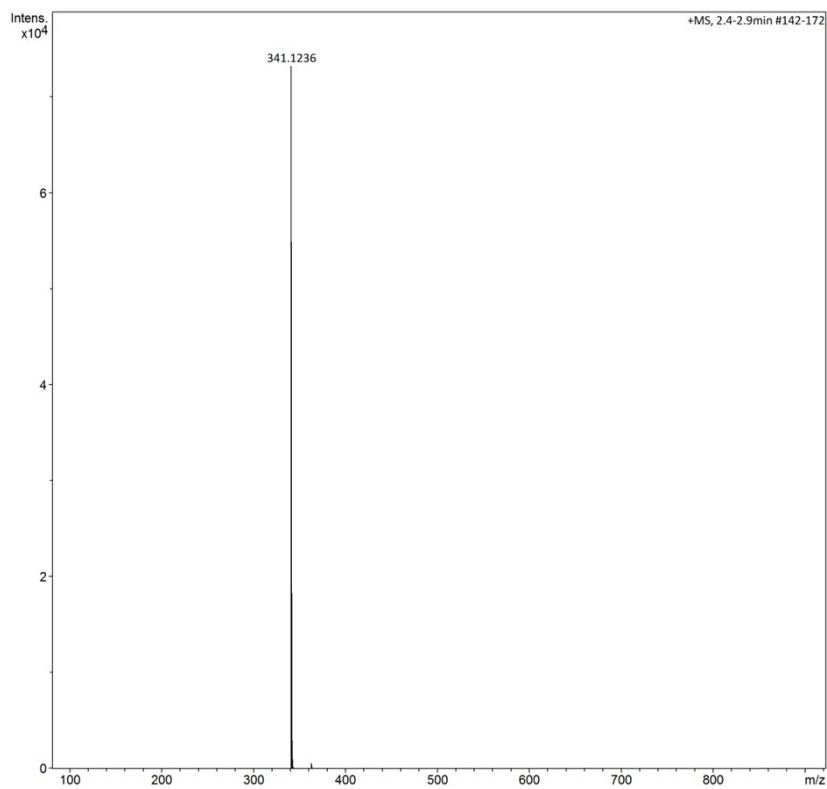
**Fig. S12.** Photobleaching experiment of probe **1c**. (a-f) The cell image of probe **1c** after laser irradiation for different times in HeLa cells. (g) average fluorescence intensity of green channel after irradiation for different times.

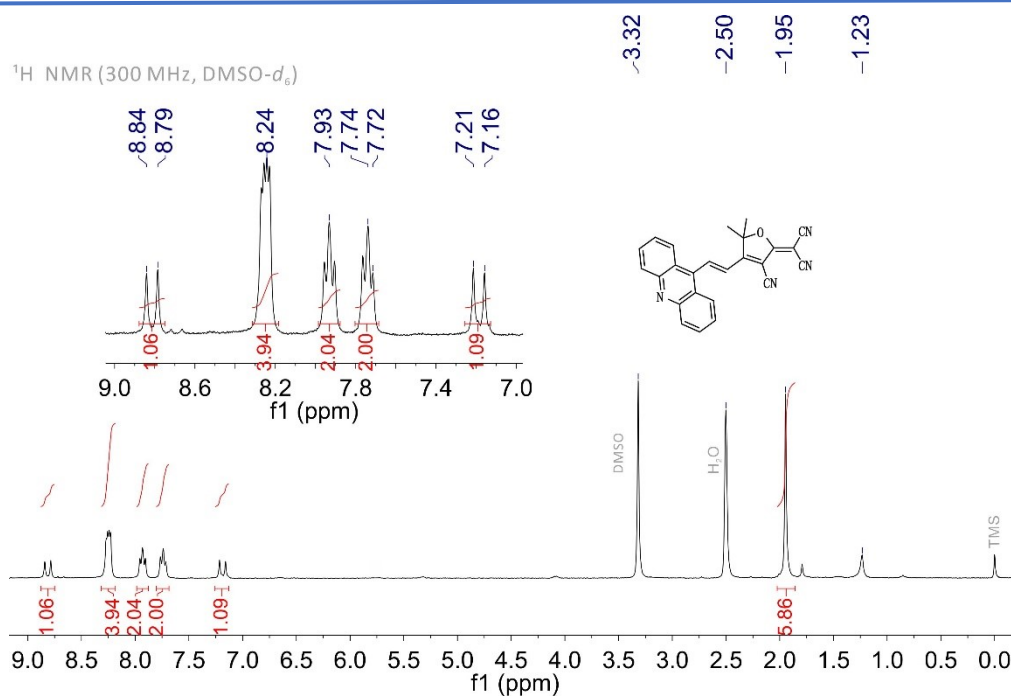
Fig. S13.  $^1\text{H}$  NMR spectrum of probe **1a**Fig. S14.  $^{13}\text{C}$  NMR spectrum of probe **1a**

Fig. S15. HRMS(ESI<sup>+</sup>) spectrum of probe 1aFig. S16 <sup>1</sup>H NMR spectrum of probe 1b

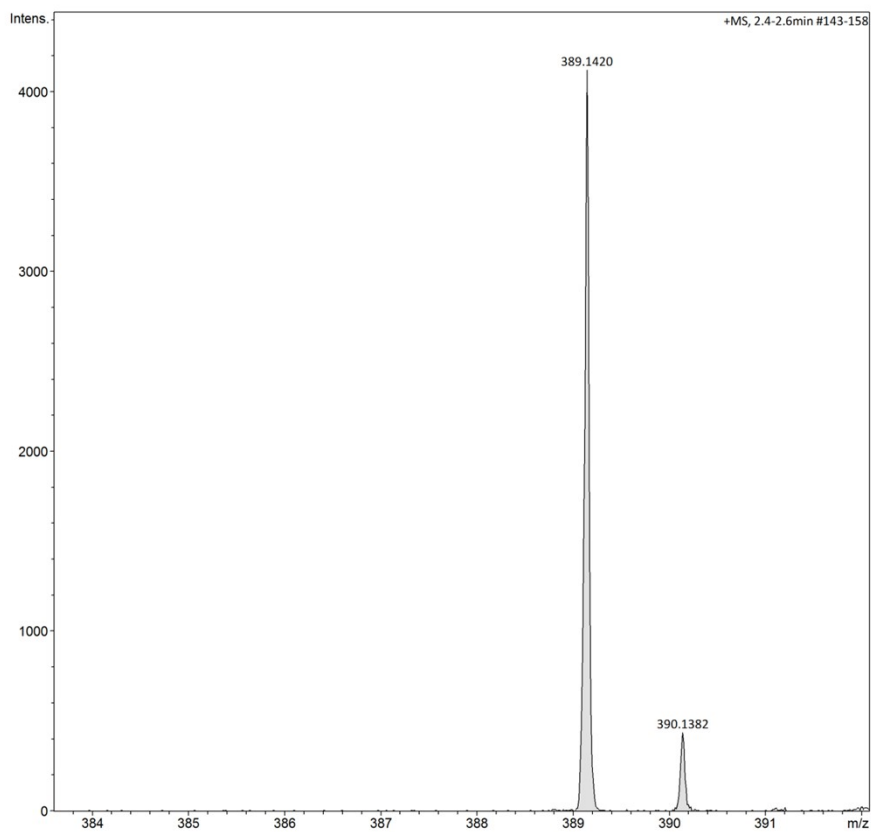
**Fig. S17** <sup>13</sup>C NMR spectrum of probe **1b**

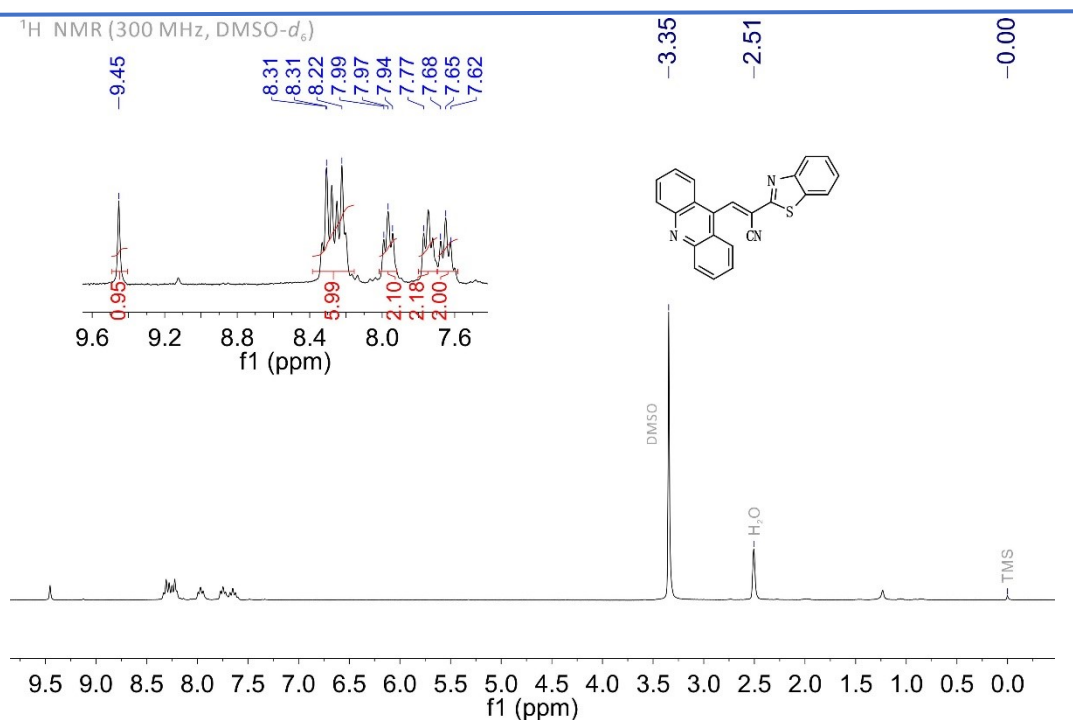
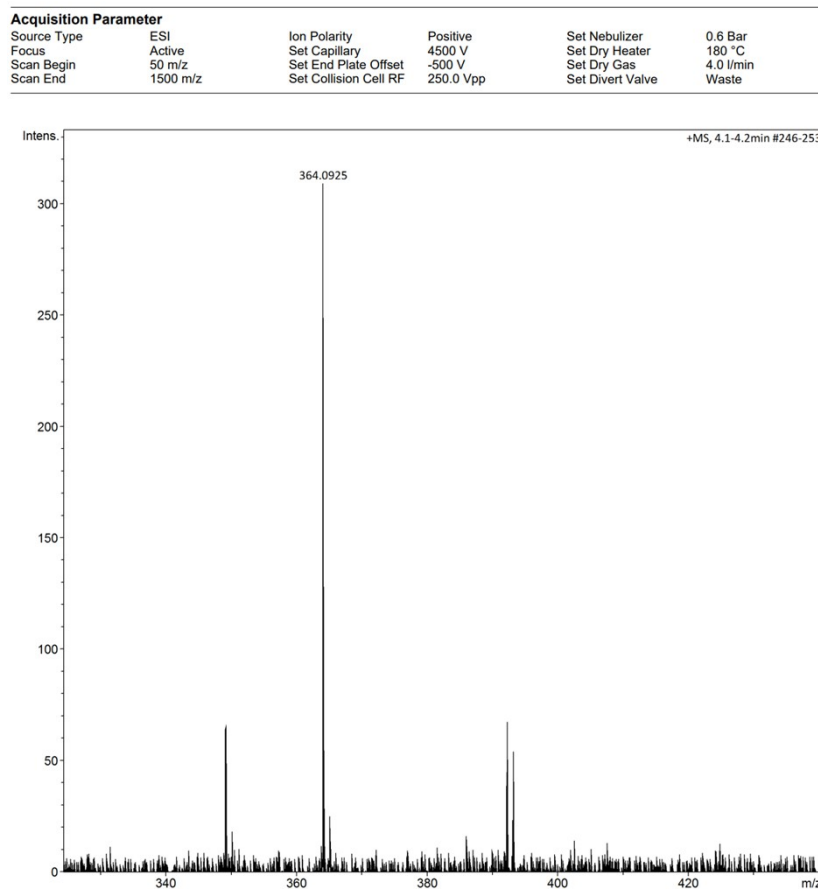
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Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

**Fig. S18** HRMS(ESI<sup>+</sup>) spectrum of probe **1b**

Fig. S19 <sup>1</sup>H NMR spectrum of probe **1c****Acquisition Parameter**

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

Fig. S20 HRMS(ESI<sup>+</sup>) spectrum of probe **1c**

Fig. S21 <sup>1</sup>H NMR spectrum of probe **1d**Fig. S22. HRMS(ESI<sup>+</sup>) spectrum of probe **1d**