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References
1. General Information

1.1 Materials
All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na₂HPO₄ water solution and 0.05mol/L KH₂PO₄ water solution with the volume ratio 4:1. Hydrazine and various analytes were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

1.2 Instruments
TLC analysis was performed using precoated silica plates. Ultraviolet–visible (UV–vis) spectra were recorded on U-3900 UV-Visible spectrophotometer. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ¹H NMR and ¹³C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI determinations were carried out on AB Triple TOF 5600plus System (AB SCIEX, Framingham, USA). HRMS determinations were carried out on an AB SCIEX TripleTOF 5600 Instrument. ESI-MS was measured with an LTQ-MS (Thermo) Instrument. The cells and zebrafish imaging experiments were measured by a Zeiss LSM880 Airyscan confocal laser scanning microscope. RGB and CIE software were downloaded from Internet.

2. Experimental Section

Scheme S1. Synthesis route of probe 1
2.1 Synthesis route of probe 1

Synthesis and Characterization of Compound 5

Compound 4 was synthesized according to our literature report (References 1). Then, compound 4 (1.485g, 5.0 mmol) and diethyl malonate (3.2 mL, 20 mmol) were dissolved in dry EtOH (60 mL) with piperidine (200 µL). The mixture was heated at 85 °C for 4 h, then cooled down to room temperature; the resulting precipitate was filtered, washed with cold ethanol and dried in vacuum, thus a desired light yellow solid was obtained (1.18 g, 60%). $^1$H NMR (600 MHz, DMSO) δ 9.16 (s, 1H), 8.96 (s, 1H), 8.78 (d, $J$ = 8.3 Hz, 1H), 8.67 (d, $J$ = 7.3 Hz, 1H), 8.07 – 8.02 (m, 1H), 4.36 (dd, $J$ = 14.2, 7.1 Hz, 2H), 4.09 – 4.03 (m, 2H), 1.64 (dt, $J$ = 15.1, 7.5 Hz, 2H), 1.37 (dt, $J$ = 14.2, 7.4 Hz, 5H), 0.94 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO) δ 163.52, 162.87, 162.78, 156.15, 155.55, 149.78, 133.62, 132.02, 130.00, 129.06, 128.94, 123.06, 120.87, 119.14, 118.63, 115.11, 61.95, 30.07, 20.27, 14.56, 14.20. ESI–MS [Compound 5 + H ]$^+$ Calcd. For: m/z Calcd 393.1, Found 394.1284.

Synthesis and Characterization of Probe 1

Compound 5 (0.786 g, 2 mmol) was suspended in 5 mL concentrated HCl and 5 mL CH$_3$COOH. The mixture was heated at 100 °C for 4 h. After the reaction was completed, it was removed from the heating bath, poured into 40 mL of ice water, and stirred for another 1 h. The precipitate was filtered, washed in cold water and dried in vacuum, thus a desired light yellow solid was obtained (0.698 g, 92%). $^1$H NMR (600 MHz, CDCl$_3$) δ 11.78 (s, 1H), 9.20 (s, 1H), 8.92 (d, $J$ = 9.4 Hz, 1H), 8.87 (d, $J$ = 7.3 Hz, 1H), 8.83 (s, 1H), 8.07 – 8.03 (m, 1H), 4.25 – 4.21 (m, 2H), 1.75 (dd, $J$ = 15.3, 7.7 Hz, 2H), 1.49 (dd, $J$ = 15.1, 7.5 Hz, 2H), 1.02 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 163.09, 162.81, 162.35, 161.51, 155.43, 151.63, 134.76, 130.76, 130.69, 129.10, 128.71, 123.37, 121.09, 115.87, 114.98, 40.69, 30.14, 20.36, 13.82. ESI–MS [Probe NC + H ]$^+$ Calcd. For: m/z Calcd 365.1, Found 366.0976.
2.2 Characterization data for synthesis.

**Figure S1**: Structure characterization of compound 5.

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\begin{align*}
\text{\textsuperscript{1}H-NMR spectrum of compound 5 in DMSO-}d_6
\end{align*}
\]

\[
\begin{align*}
\text{\textsuperscript{13}C-NMR spectrum of compound 5 in DMSO-}d_6
\end{align*}
\]
MS (ESI) spectrum of Compound 5
Figure S2: Structure characterization of probe 1.

$^1$H-NMR spectrum of Probe 1 in CDCl$_3$

$^{13}$C-NMR spectrum of Probe 1 in CDCl$_3$
Spectrum from 20170103POS-8.wiff (sample 1) - Sample006, Experiment 1, +TOF MS (100 - 2000) from 0.119 min

MS (ESI) spectrum of probe 1
Figure S3: UV-vis spectra of the probe 1 with hydrazine.

Figure S4: Kinetics study of probe 1 towards hydrazine
Figure S5: $^1$H NMR titration of probe 1 with hydrazine

$^1$H NMR comparison of probe 1 with or without hydrazine
Figure S6: The ESI-MS of product obtained by reaction of probe 1 + N$_2$H$_4$.

Figure S7: The cytotoxicity test probe 1.
Table S1: Compare of reported fluorescent probes and this work.

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<thead>
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<th>Probe</th>
<th>Detection limit</th>
<th>Response time</th>
<th>References</th>
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References