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# Electronic Supplementary Information

## A pH-sensitive near-infrared fluorescent probe with alkaline pKa for chronic wounds monitoring

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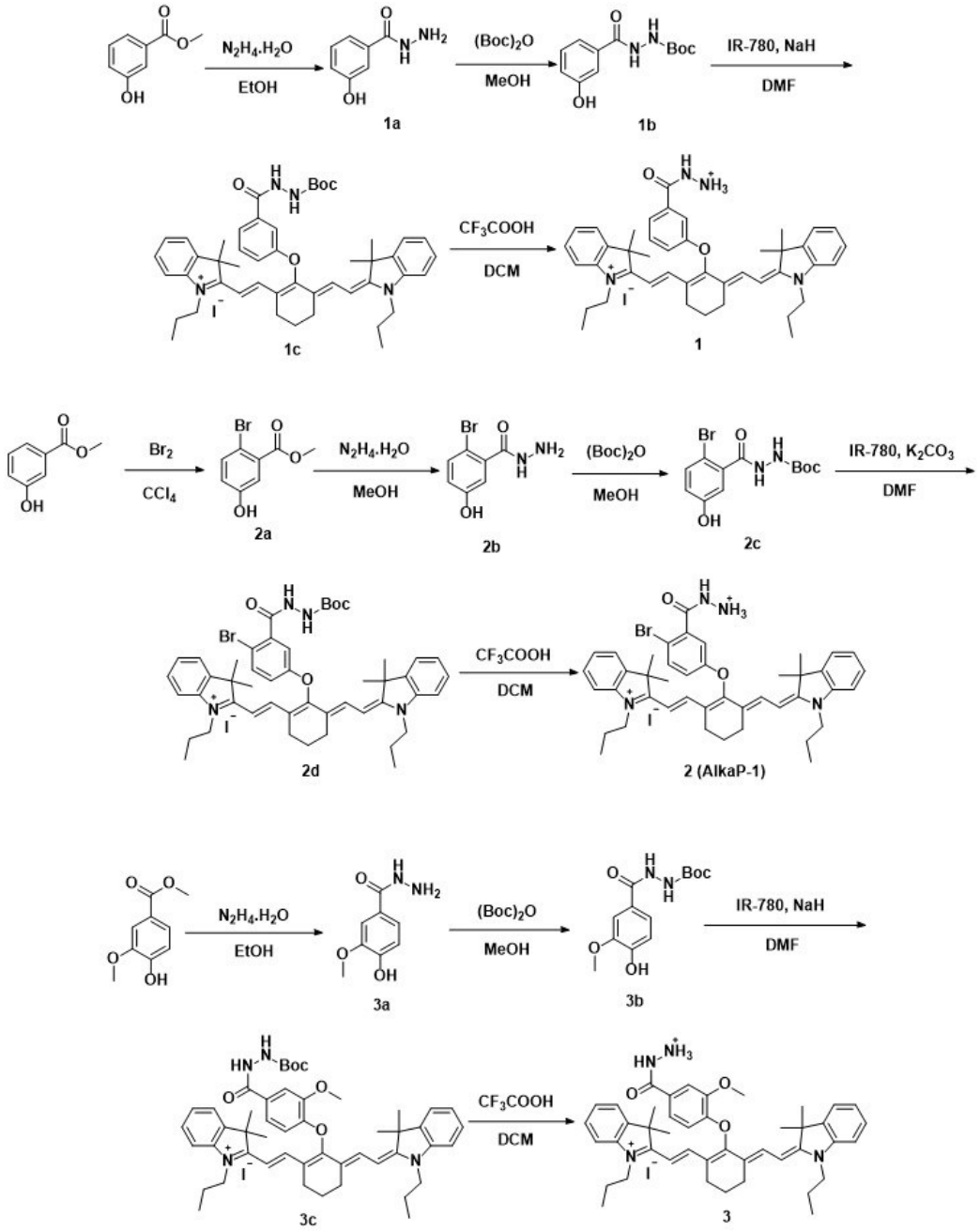
**CONTENTS**

## 1. Instruments and Materials

Unless specifically indicated, all reagents and solvents were obtained from commercial suppliers and used without further purification. IR-780 iodide[1], **1a**[2], **3a**[3] were prepared according to the literature. $^{1}H$ NMR and $^{13}C$ NMR spectra were collected by using 400 MHz Bruker spectrometer. Absorption and fluorescence spectra were conducted by using UV-Vis spectrophotometer (Shimadzu UV-2600, Japan) and fluorometer (Hitachi F-4600, Japan), respectively. Mass spectra were recorded on a Bruker TOF-MS for high-resolution mass spectra. Leica-LCS-SP8-STED was used for confocal fluorescence imaging. In vivo imaging was performed on Bruker Xtreme BI.

## 2. Synthetic routes and procedures to near-infrared compound 1, 2 and 3.




**Scheme S1.** Synthetic approach of **compound 1-3**

**Synthesis of 1b:** Di-tert-butyl dicarbonate(34 mL, 150 mmol, 1.2 eq.) was added to a solution of **1a** (19 g, 125 mmol, 1 eq.) in methanol. The resulting mixture was stirred overnight at 35 °C. After removal of excess methanol, the residue was recrystallized from dichloromethane to obtain **1b** as white crystalline solid (28 g, 89%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.09 (s, 1H), 9.72 (s, 1H), 8.87 (s, 1H), 7.53 - 6.39 (m, 4H), 1.43 (s, 9H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 166.54, 157.81, 155.94, 134.47, 129.91, 119.09, 118.25, 114.83, 79.59, 28.57. HRMS (ESI) for $C_{12}H_{16}N_2O_4$ $[M+Na]^+$: calcd 275.0955, found 275.0958.

**Synthesis of compound 1: 1b** (568 mg, 2.25 mmol, 1.5 eq.) was dissolved in anhydrous DMF(2 mL). Sodium hydride (60% in oil, 92 mg, 2.30 mmol, 1.5 eq.) was added, and the mixture was stirred for 30 min at room temperature under an argon atmosphere. IR-780 iodide (1 g, 1.50 mmol, 1 eq.) was added to the mixture, and stirring was continued overnight at 40 °C. The reaction mixture was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous $NaHCO_3$ solution twice, then dried over $Na_2SO_4$, filtered and the solvent evaporated under reduced pressure. and the residue was purified by column chromatography ($CH_2Cl_2$/MeOH, 30 : 1) to afford the crude product **1c** as green solid. Trifluoroacetic acid(10 mL) was added to a solution of the crude product **1c** in dichloromethane. The resulting mixture was stirred at 30 °C for 2 h and then remove excess trifluoroacetic acid and dichloromethane, the residue was purified by column chromatography ($CH_2Cl_2$/MeOH, 10 : 1) to afford compound **1** as green solid(0.71 g, 53%). $^{1}$H NMR (400 MHz, MeOD) $\delta$ 7.97 (d, $J$ = 14.2 Hz, 2H), 7.65 (s, 1H), 7.55 - 7.52 (m, 2H), 7.42 - 7.34 (m, 5H), 7.31 - 7.15 (m, 4H), 6.19 (d, $J$ = 14.2 Hz, 2H), 4.09 (t, $J$ = 7.2 Hz, 4H), 2.77 (t, $J$ = 5.5 Hz, 4H), 2.07 (m, 2H), 1.84 (m, 4H), 1.34 (s, 12H), 1.03 (t, $J$ = 7.4 Hz, 6H). $^{13}$C NMR (101 MHz, MeOD) $\delta$ 172.41, 167.11, 163.17, 159.94, 142.27, 141.60, 141.04, 130.52, 128.39, 124.87, 121.97, 121.54, 120.70, 118.02, 113.56, 110.72, 99.85, 53.47, 48.88, 45.10, 26.81, 23.81, 20.96, 20.39, 10.26. HRMS (ESI) for $C_{43}H_{52}N_4O_2^{2+}$: calcd 328.2041, found 328.2037.

**Synthesis of 2a:** Methyl 3-hydroxybenzoate (2 g, 13.1 mmol, 1 eq.) was placed in a round bottom flask and dissolved in tetrachloromethane (8 mL). Then bromine (0.7 mL, 27.3 mmol, 2 eq.) was added and the solution was stirred overnight at 50 °C. The resulting solution was cooled under 0 °C for 2 h and filtered in vacuum to obtain the crude product. The crude product was recrystallized from tetrachloromethane to obtain compound **2a** as white crystalline solid (2.1 g, 69%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.12 (s, 1H), 7.50 (d, $J$ = 8.7 Hz, 1H), 7.15 (d, $J$ = 3.0 Hz, 1H), 6.89 (dd, $J$ = 8.7, 3.0 Hz, 1H), 3.83 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 166.49, 157.21, 135.21, 133.34, 120.77, 117.94, 108.76, 52.94. HRMS (ESI) for $C_8H_7BrO_3$ $[M+Na]^+$: calcd 254.9431, found 254.9444.

**Synthesis of 2b:** Under an argon atmosphere, an 85% aqueous solution of hydrazinehydrate (21.7 g, 434 mmol, 15 eq.) was added to a solution of **2a** (6.7 g, 29 mmol, 1 eq.) in methanol (15 mL).The reaction mixture was heated at 60 °C for 5 h. After removal of excess hydrazine hydrate and methanol, water was added and the precipitated product **2a** was filtered as white solid(5.4 g, 80%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.92 (s, 1H), 9.49 (s, 1H), 7.40 (d, $J$ = 8.5 Hz, 1H), 6.76 (dd, $J$ = 8.5, 2.8Hz, 1H), 6.73 (d, $J$ = 2.8 Hz, 1H), 4.46 (s, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 166.97, 157.03, 138.94, 133.99, 118.51, 116.47, 108.04. HRMS (ESI) for $C_7H_7BrN_2O_2$ $[M+Na]^+$: calcd

254.9519, found 254.9541.

**Synthesis of 2c:** Di-tert-butyl dicarbonate(2.4 mL, 10.4 mmol, 1.2 eq.) was added to a solution of **2b** (2 g, 8.6 mmol, 1 eq.) in methanol. The resulting mixture was stirred overnight at 35 °C. After removal of excess methanol, the residue was recrystallized from dichloromethane to obtain the compound **2c** as white crystalline solid (2.6 g, 90%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.01 (s, 1H), 9.96 (s, 1H), 9.02 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 6.86 (d, *J* = 2.7Hz, 1H), 6.79 (dd, *J* = 8.7, 2.7 Hz, 1H), 1.43 (s, 9H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 167.19, 157.04, 155.58, 137.91, 134.24, 119.00, 116.74, 107.81, 79.67, 28.57. HRMS (ESI) for $C_{12}H_{15}BrN_2O_4$ $[M+Na]^+$: calcd 354.9923, found 354.9974.

**Synthesis of compound 2 (AlkaP-1) :** Under an argon atmosphere, IR-780 iodide (2 g, 3 mmol, 1 eq.) was added to a solution of **2c** (1.5 g, 4.5 mmol, 1.5 eq.) and $K_2CO_3$(0.62 g, 4.5 mmol, 1.5 eq.) in anhydrous DMF (10 mL).The reaction mixture was stirred at 40 °C for 4 h and then dissolved in ethyl acetate (100 mL). Organic phase was washed with saturated aqueous $NaHCO_3$ solution twice and dried over $Na_2SO_4$, filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using methanol/dichloromethane (1:10) as eluent to obtain the crude product **2d** as green solid. Trifluoroacetic acid(10 mL) was added to a solution of the crude product **2d** in dichloromethane. The resulting mixture was stirred at 30 °C for 2 h and then remove excess trifluoroacetic acid and dichloromethane, the residue was purified by column chromatography using methanol/dichloromethane (1 : 10) as eluent to obtain compound **2** as green solid(1.75 g, 60%). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.86 (d, *J* = 14.1 Hz, 2H), 7.57 (d, *J* = 8.8 Hz, 1H), 7.34 (m, 4H), 7.25 (d, *J* = 2.8 Hz, 1H), 7.20 (t, 2H), 7.08 - 7.01 (m, 3H), 6.01 (d, *J* = 14.2 Hz, 2H), 3.98 (t, 4H), 2.68 (t, 4H), 2.04 (m, 2H), 1.86 (m, 4H), 1.41 (s, 12H), 1.05 (t, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 172.37, 165.82, 163.08, 158.48, 142.14, 141.63, 141.15, 138.45, 134.90, 128.53, 125.19, 122.45, 121.71, 117.50, 116.24, 112.66, 110.50, 100.15, 77.31, 49.21, 45.87, 29.67, 27.96, 24.28, 20.96, 20.75, 11.60. HRMS (ESI) for $C_{43}H_{51}BrN_4O_2^{2+}$: calcd 367.1592, found 367.1596.

**Synthesis of 3b:** Di-tert-butyl dicarbonate (24.4 mL, 100 mmol, 1.2 eq.) was added to a solution of **3a** (16 g, 90 mmol, 1 eq.) in methanol. The resulting mixture was stirred overnight at 35 °C. After removal of excess methanol, the residue was recrystallized from dichloromethane to obtain **3b** as white crystalline solid (23 g, 93%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.98 (s, 1H), 9.69 (s, 1H), 8.83 (s, 1H), 7.45 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 1H), 3.82 (s, 3H), 1.43 (s, 9H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 166.11, 156.11, 150.39, 147.63, 123.86, 121.52, 115.38, 111.71, 79.53, 56.08, 28.57. HRMS (ESI) for $C_{13}H_{18}N_2O_5$ $[M+Na]^+$: calcd 305.1050, found 305.1047.

**Synthesis of 3: 3b** (635 mg, 2.25 mmol, 1.5 eq.) was dissolved in anhydrous DMF(2 mL). Sodium hydride (60% in oil, 92 mg, 2.30 mmol, 1.5 eq.) was added, and the mixture was stirred for 30 min at room temperature under an argon atmosphere. IR-780 iodide (1 g, 1.50 mmol, 1 eq.) was added to the mixture, and stirring was continued overnight at 40 °C. The reaction mixture was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous $NaHCO_3$ solution twice, then dried over $Na_2SO_4$, filtered and the solvent evaporated under reduced pressure. and the residue was purified by column chromatography ($CH_2Cl_2$/MeOH, 30 : 1) to afford the crude

product **3c** as green solid. To the crude product **3c** was added trifluoroacetic acid (10 mL) and stirred at 30 °C for 2 h, then remove the solvent and the residue was purified by column chromatography ($CH_2Cl_2$/MeOH, 10 : 1) to afford compound **3** as green solid (0.74 g, 58%). $^1$H NMR (400 MHz, MeOD) $\delta$ 7.93 (d, *J* = 14.2 Hz, 2H), 7.74 (d, *J* = 1.0 Hz, 1H), 7.48 - 7.14 (m, 9H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.19 (d, *J* = 14.2 Hz, 2H), 4.18 (s, 3H), 4.10 (t, *J* = 7.1 Hz, 4H), 2.76 (t, 4H), 2.06 (m, 2H), 1.84 (m, 4H), 1.37 (s, 12H), 1.03 (t, *J* = 7.4 Hz, 6H). $^{13}$C NMR (101 MHz, MeOD) $\delta$ 172.42, 163.70, 151.21, 148.22, 142.29, 141.40, 141.04, 128.40, 127.30, 124.88, 122.02, 121.46, 120.36, 113.48, 111.44, 110.74, 99.88, 55.61, 53.48, 48.91, 45.12, 26.76, 23.83, 21.00, 20.40, 10.25. HRMS (ESI) for $C_{44}H_{54}N_4O_3^{2+}$: calcd 343.2088, found 343.2089.

**3. Optical Measurement**

All the absorbance and fluorescence spectra were obtained at room temperature with increment set to 1 nm. All solutions were prepared freshly for each measurement. Citrate-phosphate buffer was used for preparation from pH 4.5 to pH 8.5, while carbonate-bicarbonate buffer was made for pH 9.0 to pH 10.5. Unless specifically indicated, probe at 10 μM concentration was prepared for all optical measurements. Fluorescence spectra were collected at the excitation wavelengths of 715 nm with increments of 1 nm, respectively. Unless specifically indicated, slit widths were set to 10 nm for all emission measurements.

**4. Absorbance and Fluorescence spectra of compound 1 and 3.**

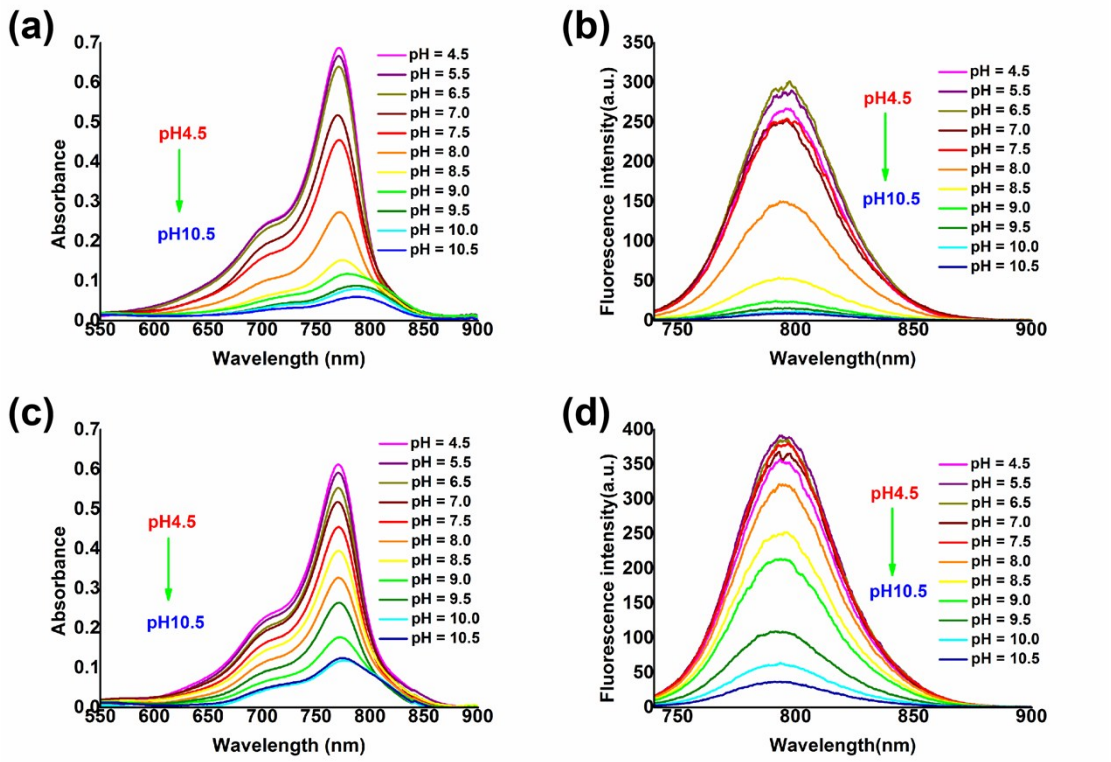



**Figure S1.** Absorbance spectra of 5 μM (a) compound **2** and (c) compound **3** with different pH buffers containing 10 % DMSO, respectively; Fluorescence spectra of 10 μM (b) compound **2** and (d) compound **3** with different pH buffers containing 10 % DMSO at excitation of 715 nm, respectively.

**5. Determination of $pK_a$ by fluorometric titration**

The constant $K_a$ of probes were determined in buffer solutions by fluorometric titration as a function of pH using the fluorescence spectra. The expression of the steady-state fluorescence intensity $F$ as a function of the proton concentration has been extended for the case of a n : 1 complex between $H^+$ and a fluorescent probe, which is expressed by the equation below: [6]

$$F = \frac{F_{\min}\left[H^+\right]^n + F_{\max}K_a}{\mathrm{K_a} + \left[H^+\right]^n}$$

$F_{\min}$ and $F_{\max}$ stand for the fluorescence intensities at maximal and minimal $H^+$ concentrations, respectively, and n is apparent stoichiometry of $H^+$ binding to the probe which affects the fluorescent change. Nonlinear fitting of equation expressed above to the fluorescence titration data recoded as a function of $H^+$ concentration with $K_a$ and n as free adjustable parameters yields the estimated apparent constant of $K_a$.

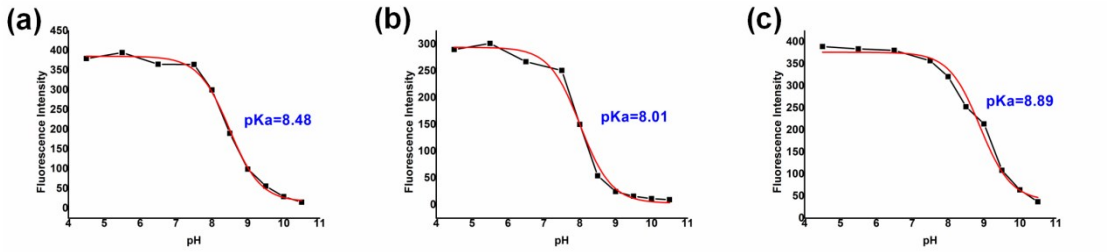



**Figure S2.** Fluorescence intensity at 798 nm of **compound 1** (a), **compound 2** (**AlkaP-1**, b) and **compound 3** (c) versus pH under excitation at 715 nm, respectively.

**6. Calculation of fluorescence quantum yield**

The quantum yields were calculated using the equation reported[7] and cyanine dye IR-780 iodide was used as a standard with fluorescence quantum yield of 7.6 % in methanol.[8] Sample solutions and standard solutions with absorbance values below 0.05 were freshly made, with excitation wavelengths all set to 715 nm and slit widths set to 10 and 20 nm. Integrated fluorescence intensity in the range from 750 to 900 nm was obtained by employing OriginPro 9.0 software.

$$\Phi_{sample} = \Phi_{reference} \times \frac{slope_{sample}}{slope_{reference}} \times \frac{\eta^2_{sample}}{\eta^2_{reference}}$$

Where $\Phi$ is the fluorescence quantum yield, the subscripts '*sample*' and '*reference*' stand for test and standard , respectively, "*slope*" represents the gradient from the plot of integrated fluorescence intensity versus absorbance and $\eta$ is the refractive index of the solvent.

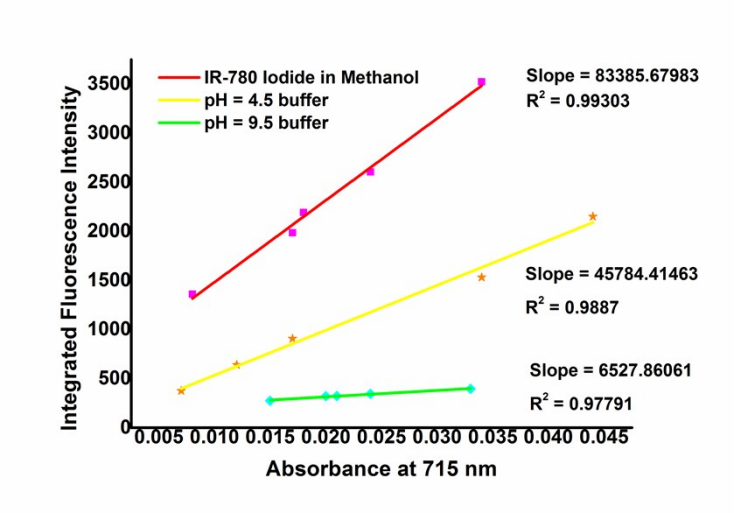



**Figure S3.** Quantum yield Measurements, using IR-780 in methanol as a standard, of **AlkaP-1** in pH 4.5 and 9.5 buffers, respectively. Five different concentrations were measured and the tendency chart of integrated fluorescence intensity versus absorbance was ploted.

## 7. Dye stability and selectivity

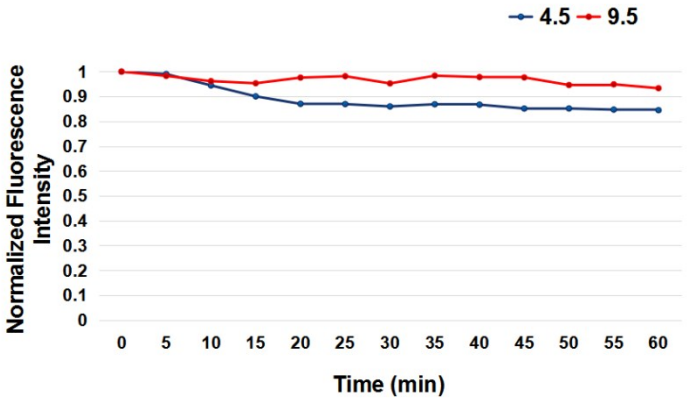
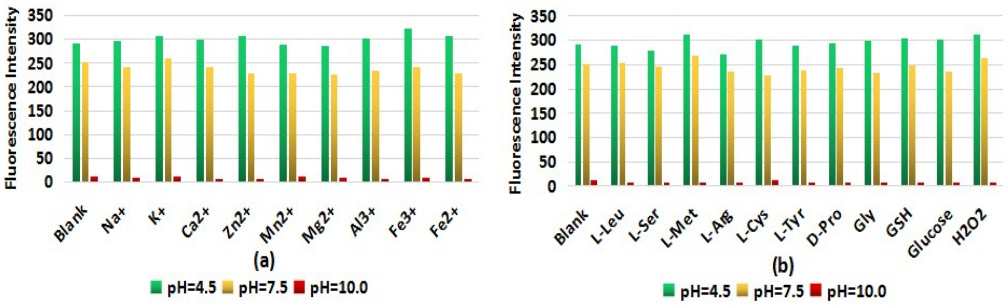



**Figure S4.** Fluorescence intensity of **AlkaP-1** versus excitation time in pH 4.5 and 9.5 buffers, respectively.



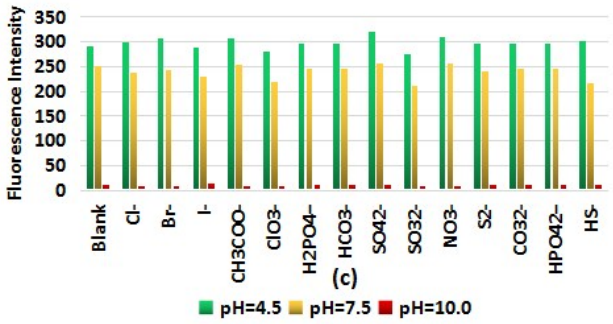



**Figure S5.** Fluorescence responses of **AlkaP-1** to different metal cations (a), amino acids (b) and anions (c) (50 μM) in pH 4.5, 7.5, and 10.0 buffers, respectively, under excitation of 715 nm.

**8. Cytotoxicity of the probe**

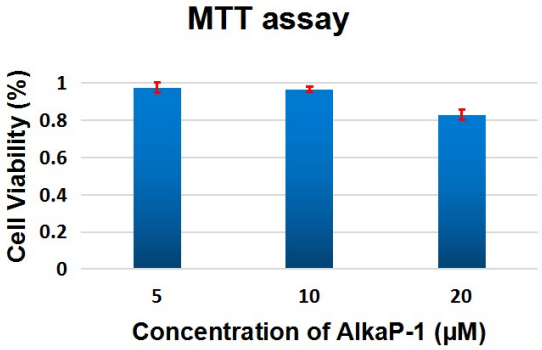



**Figure S6.** Cytotoxicity of **AlkaP-1** through standard MTT assay by incubation of Ewing's Sarcoma cells A673 with 5, 10, 20 μM of **AlkaP-1** for 24 hours, respectively. The cell viability is directly related to the absorbance at 450 nm. The error bars indicate ± SD.

**9. Cell culture and fluorescence Live Cell Imaging**

**Cell Culture and Cytotoxicity Assay.** Ewing's Sarcoma cells A673 were purchased from ATCC (Mannassas, VA) and were cultured in Dulbecco's Modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, fisher Scientific) at 37 °C in humidified air and 5% $CO_2$. The cells were subcultured at 90% confluence with 0.25% trypsin (w/v) every 2-3 days. Standard Cell Counting Kit-8 assay (CCK-8, MedChem Express, NJ, USA) was used to investigate the cytotoxicity against Ewing's Sarcoma cells. The cells were seeded in 96-well plates at an initial density of 3000 cells per well, with 100 μL DMEM medium per well. After seeding 24 hours on the 96 well plate, the medium was replaced by different concentrations of **AlkaP-1** (0, 5, 10, 20 μM concentration solutions in fresh culture medium, 100 μL/well) for 24 h at 37 °C under 5% $CO_2$. Added 10 μL of the CCK-8 solution to each well of the plate. After incubating for another 1 h, the cell viability was determined by measuring the light absorbance at 450 nm with a microplate reader (BioTek ELx800). Untreated cells were employed as controls. Percent (%) cell viability was calculated by comparing the absorbance of the control cells to that of treated cells. Data were summarized as a plot where each data point represents an average of three wells.

**Fluorescence live cell imaging.** For confocal live cell imaging, Ewing's Sarcoma cells were seeded into the 35 nm glass-bottom culture dishes (MatTek, MA) and allowed 1 day to reach 50% confluence. After 24 hours of incubation, the cell culture medium was replaced by freshly prepared FBS-free medium with 5, 10, 15, and 20 μM of **AlkaP-1** for 1 hour at 37 °C under 5% $CO_2$ followed by using PBS buffer to rinse three times. For the live cell fluorescence imaging at different pH, the Ewing's Sarcoma cells were incubated with 15 μM **AlkaP-1** at 37 °C under 5% $CO_2$ for 1 hour. The cells were rinsed with PBS buffer before they were incubated with high $K^+$ buffer (30 mM NaCl, 120 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$, 1 mM $NaH_2PO_4$, 5 mM glucose, 20 mM HEPES, and 20 mM NaOAc) at various pH values (4.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5) in the presence of 15 μg/mL of nigericin for 30 min to equilibrate the intracellular and extracellular pH, then imaging with an excitation filter 720 nm and emission channel 800 nm.

**10. Fluorescence imaging and wound pH monitoring in diabetic mouse.**

All animal imaging experiments were performed on BKS-DB male mouse. BKS-DB diabetic mice (6 weeks old) were purchased from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). All animal operations were in accord with institutional animal use and care regulations. To avoid occasionality, in this animal experiment, we use three mice to perform the parallel experiment. Similar wounding of mice was performed as described previously.[9] The hair on the back of mice was shaved and six full-thickness excisional wounds (8 mm in diameter) were made on the back of mice. To assess the pH changes during the course of delayed wound healing, we choose three of those wounds and set three time points (days 1, 4, 7) to record the fluorescence intensity, which were represented by the pseudocolor images. At each time point, diabetic mice were narcotized with intraperitoneal injection of pentobarbital and one of the wounds on the back of mice was sprayed with 15 μM **AlkaP-1** in normal saline containing 10% DMSO. After 1 h, the wound was rinsed three times with normal saline, then imaging. The mice were imaged with an excitation filter 720 nm and emission channel 790 nm.

As for the measurement of the surface pH values of the wound bed, a commercial at glass pH electrode (BPH-600, Qingdao Lubo Weiye Environmental Technology Co., Ltd. China) was used. At each time point pH values of three wound points at each mouse were measured with the electrode. Data were summarized as a plot where each data point represents an average of 9 points.

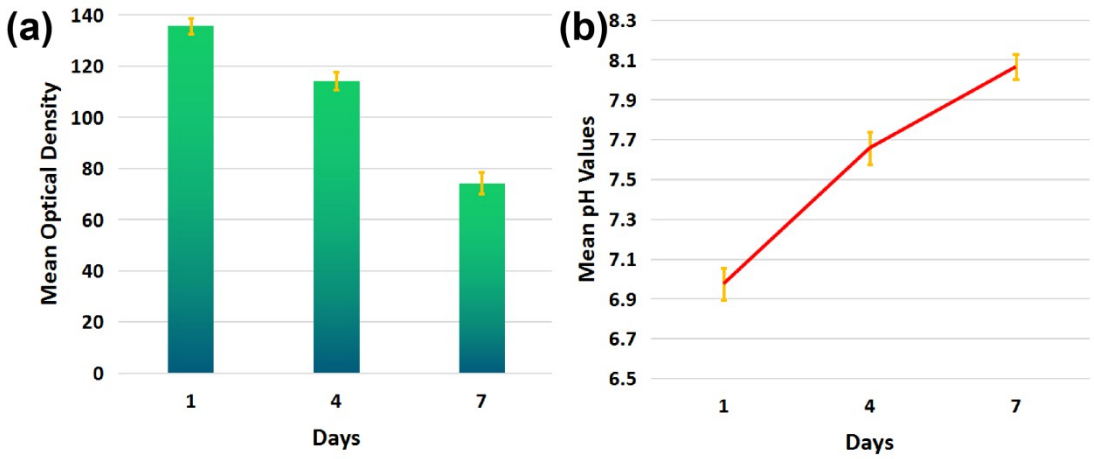



**Figure S7.** (a) Semiquantitative analysis to fluorescence intensity of each wound was obtained by employing imaging system analysis software and presented as mean optical density ± SD, with n = 3. (b) Wound pHs at different time points (days 1, 4 and 7) were measured with the pH electrode and each data point of wound pH in the plot presented as mean ± SD, with n = 9.

### 11. $^{1}H$, $^{13}C$ NMR and HRMS spectra of all the synthetic compounds

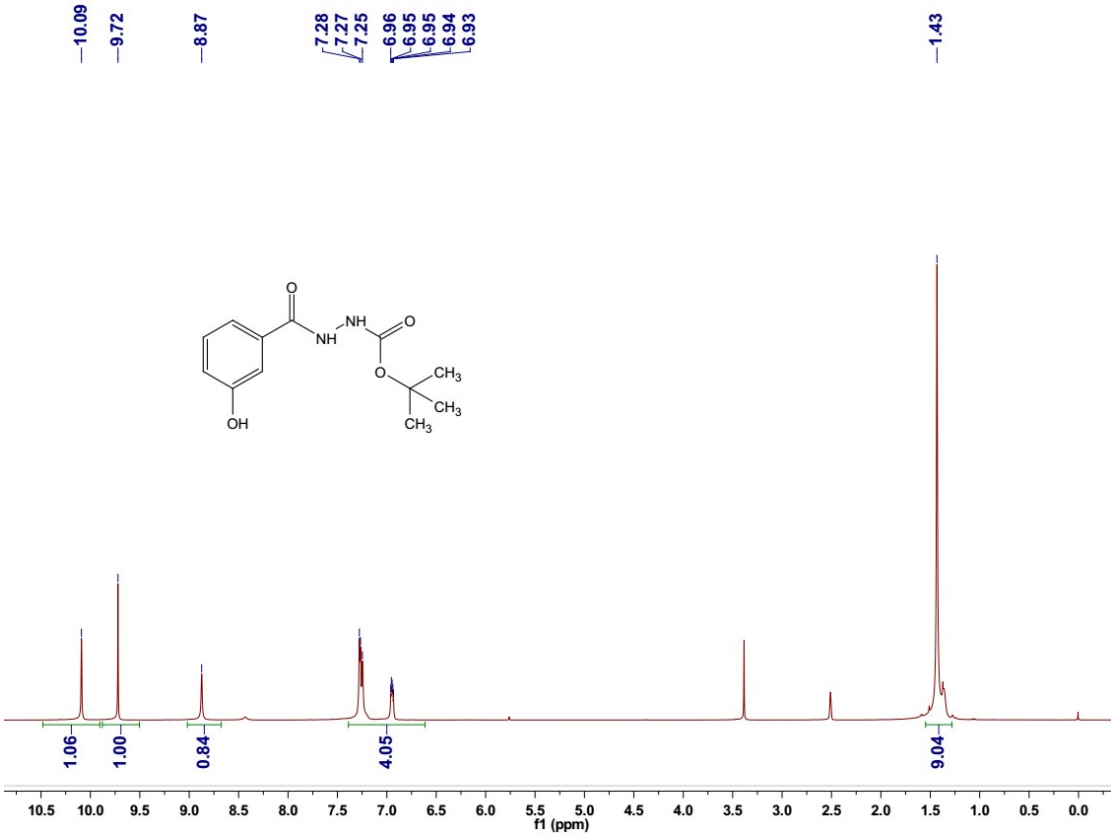



**Figure S8.** $^{1}H$ NMR spectrum of **1b** in DMSO-$d_6$ solution.

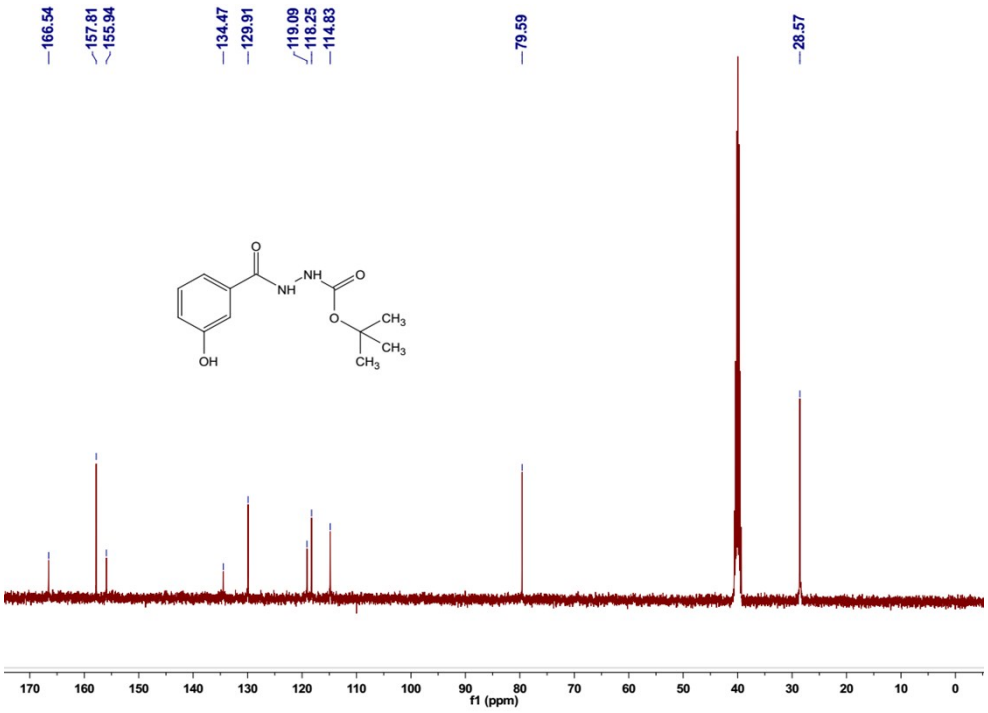



**Figure S9.** $^{13}$C NMR spectrum of **1b** in DMSO-$d_6$ solution

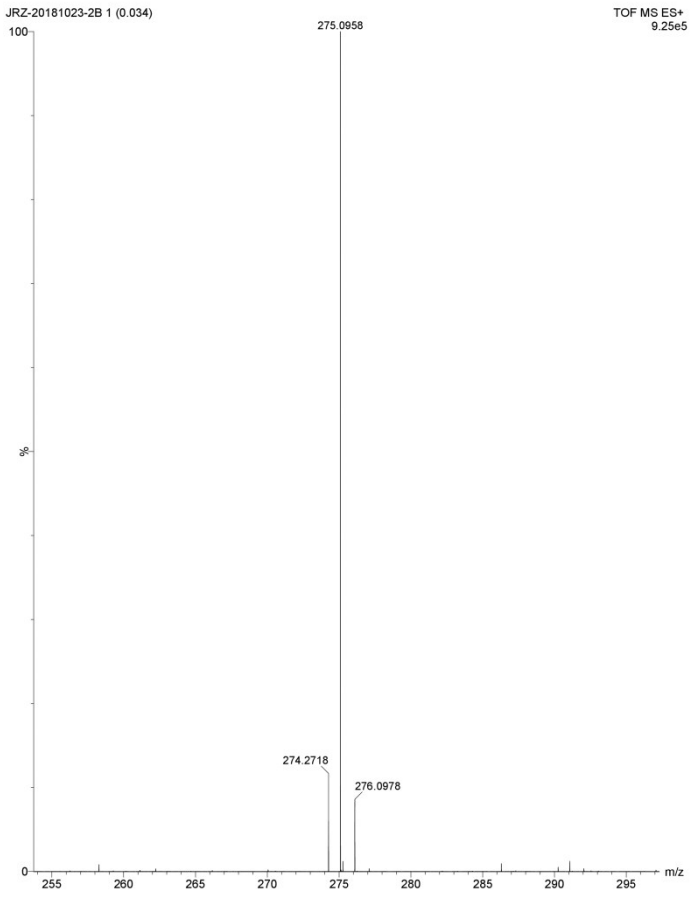



**Figure S10.** High resolution mass spectrometry spectrum of **1b**.

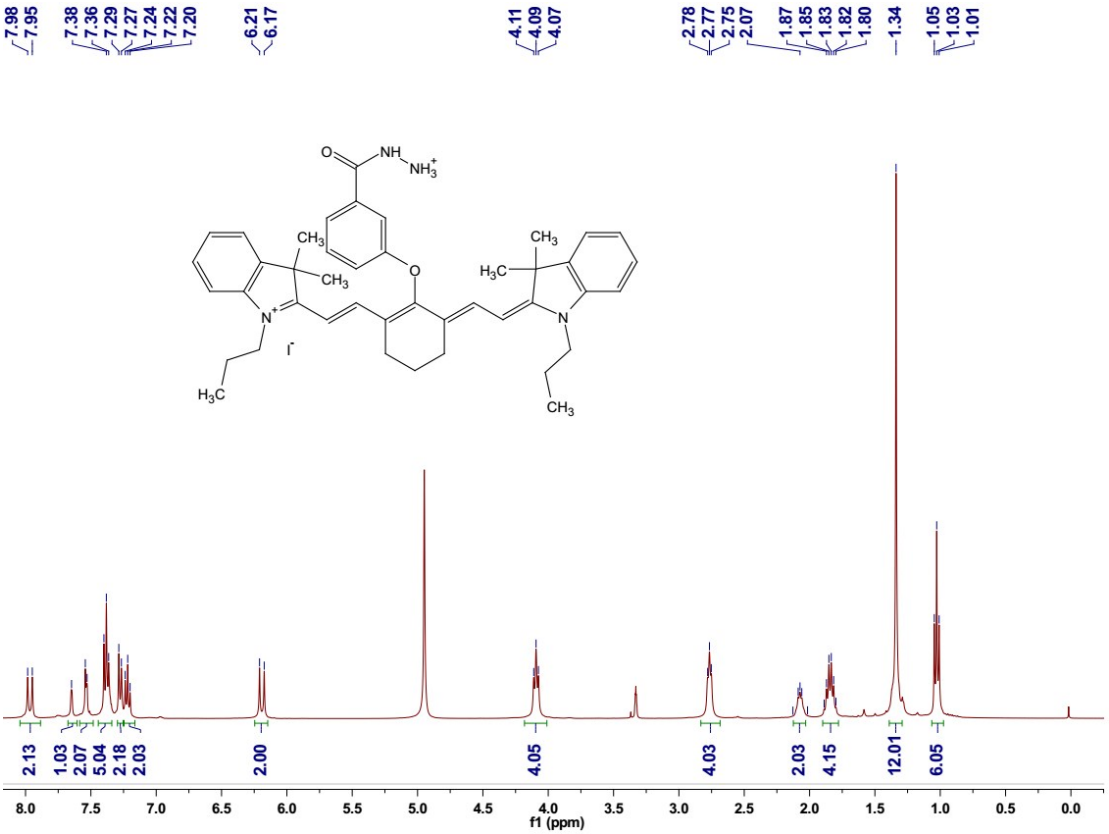



**Figure S11.** $^{1}$H NMR spectrum of **compound 1** in $CD_3OD$ solution.

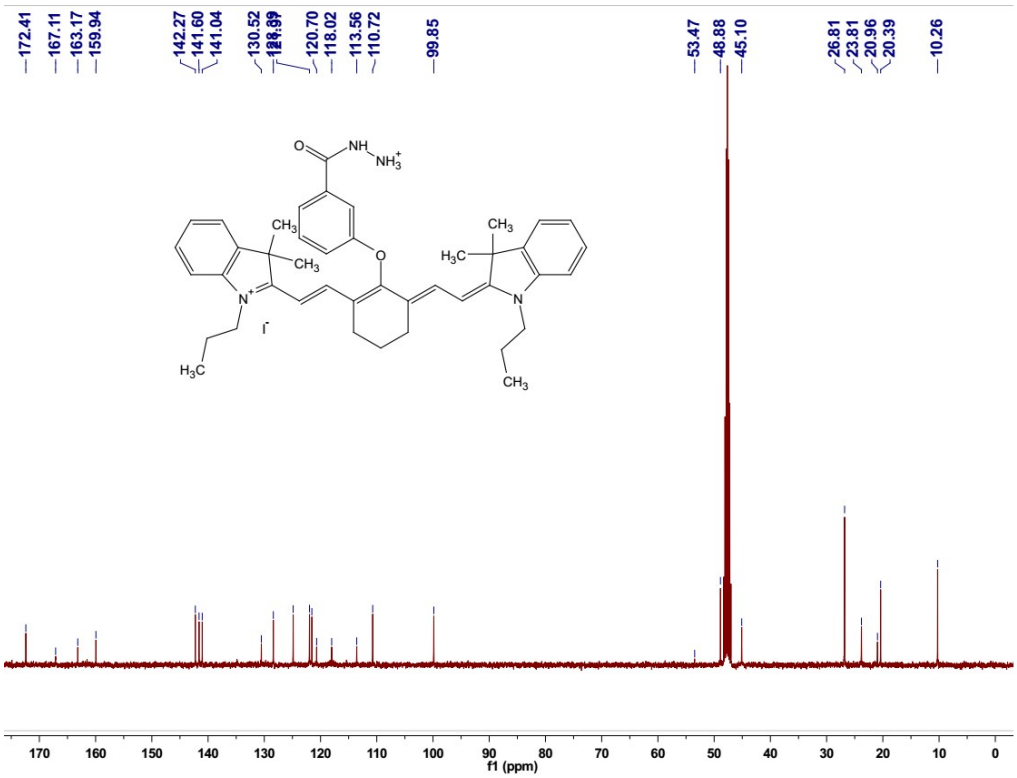



**Figure S12.** $^{13}$C NMR spectrum of **compound 1** in $CD_3OD$ solution

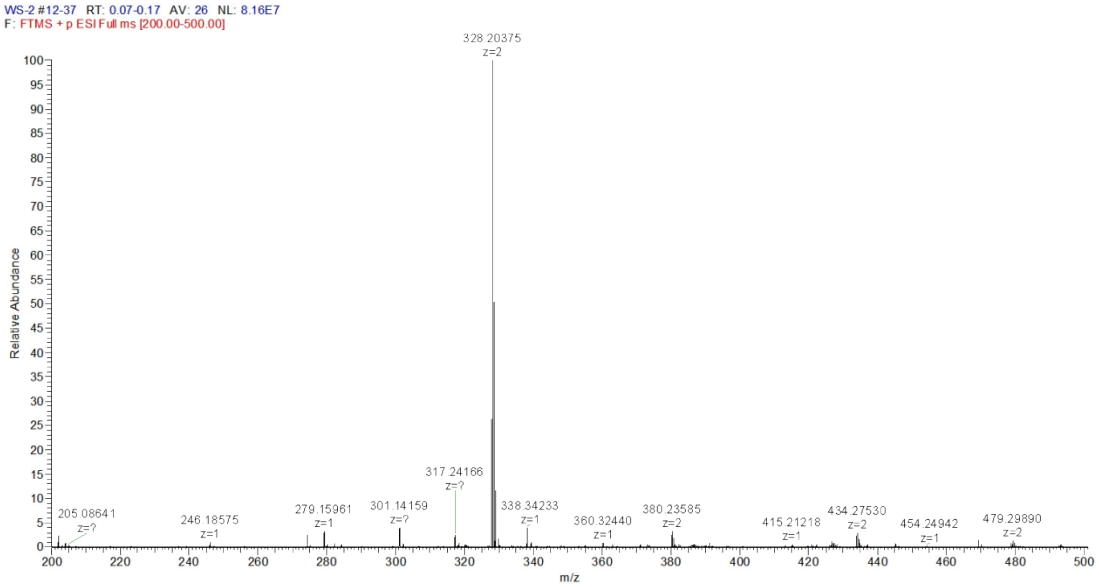



**Figure S13.** High resolution mass spectrum of **compound 1**.

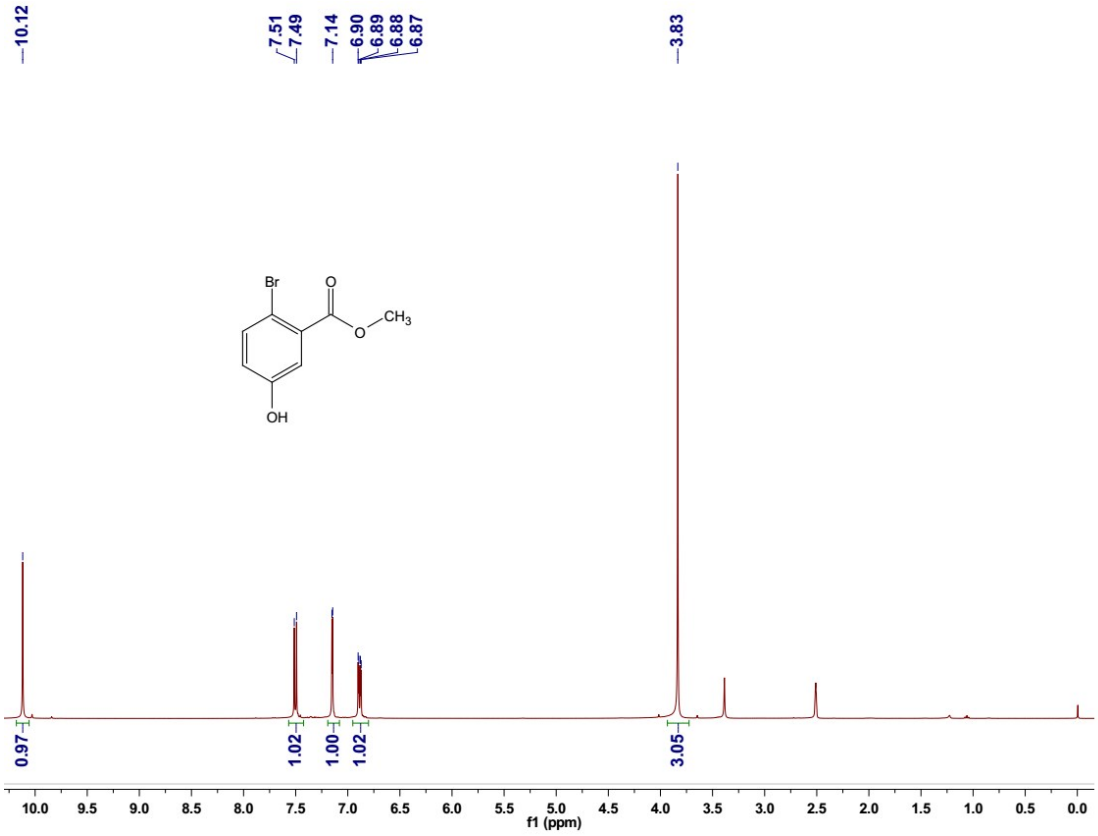



**Figure S14.** $^{1}$H NMR spectrum of **2a** in DMSO-$d_6$ solution.

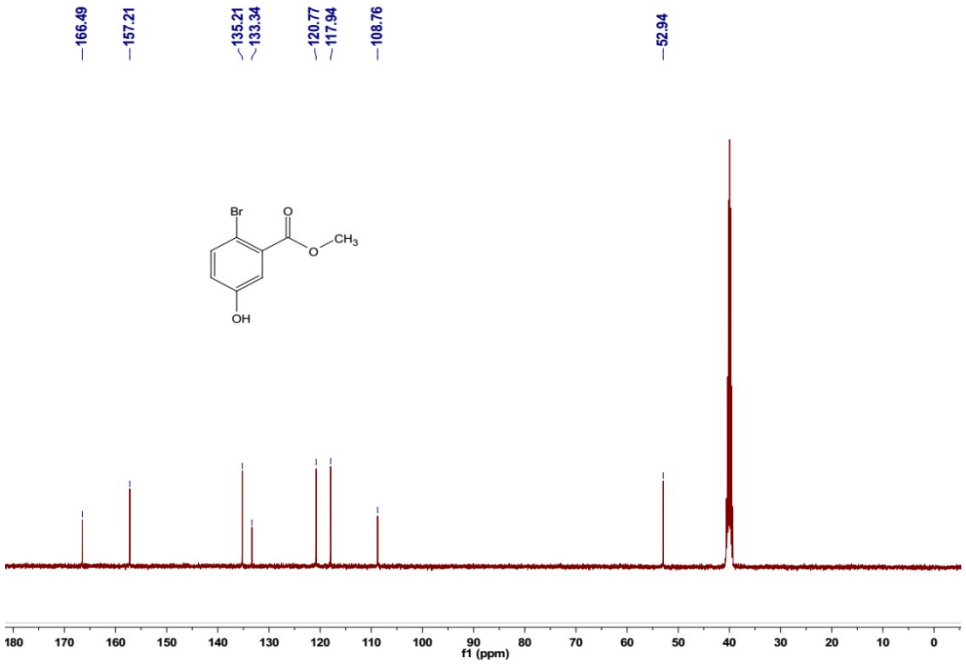



**Figure S15.** $^{13}C$ NMR spectrum of **2a** in DMSO-$d_6$ solution

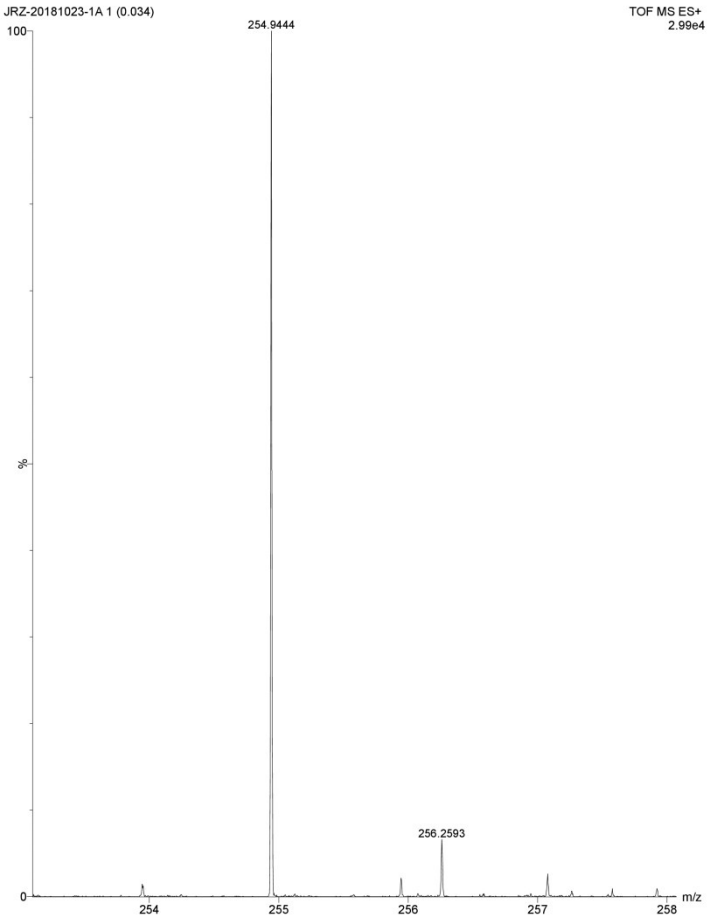



**Figure S16.** High resolution mass spectrum of **2a**.

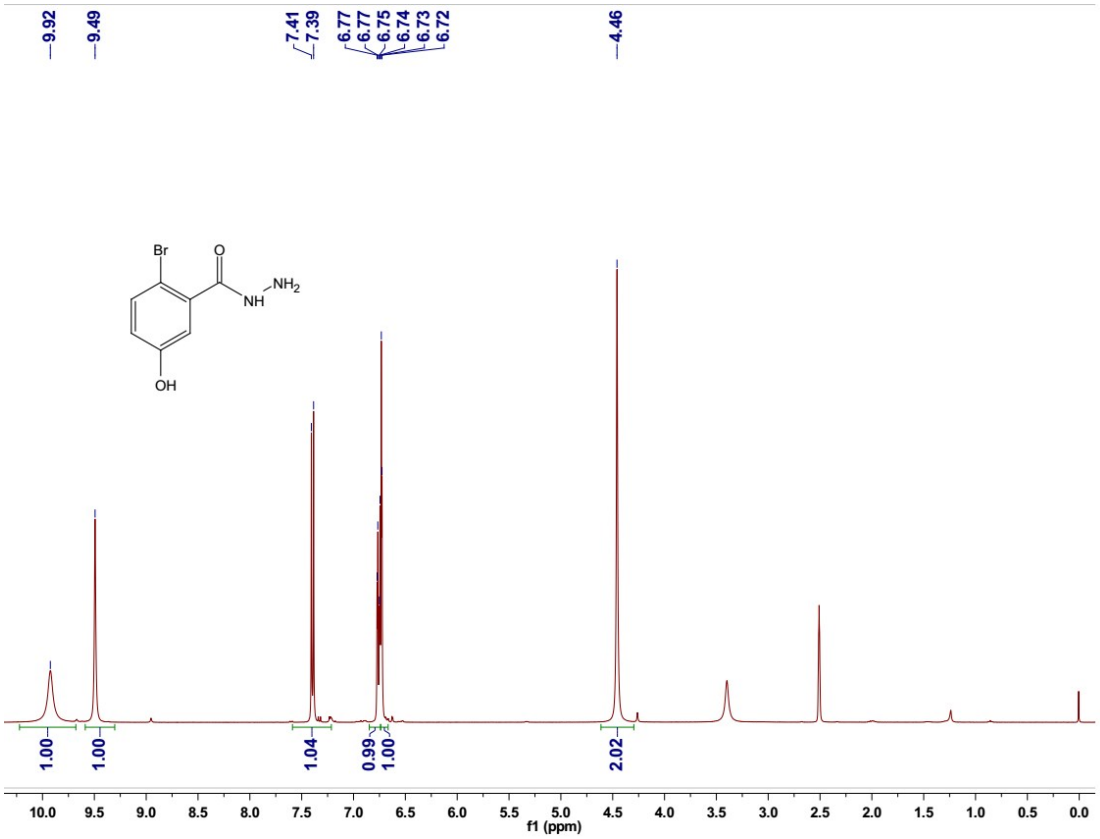



**Figure S17.** $^{1}$H NMR spectrum of **2b** in DMSO-$d_6$ solution.

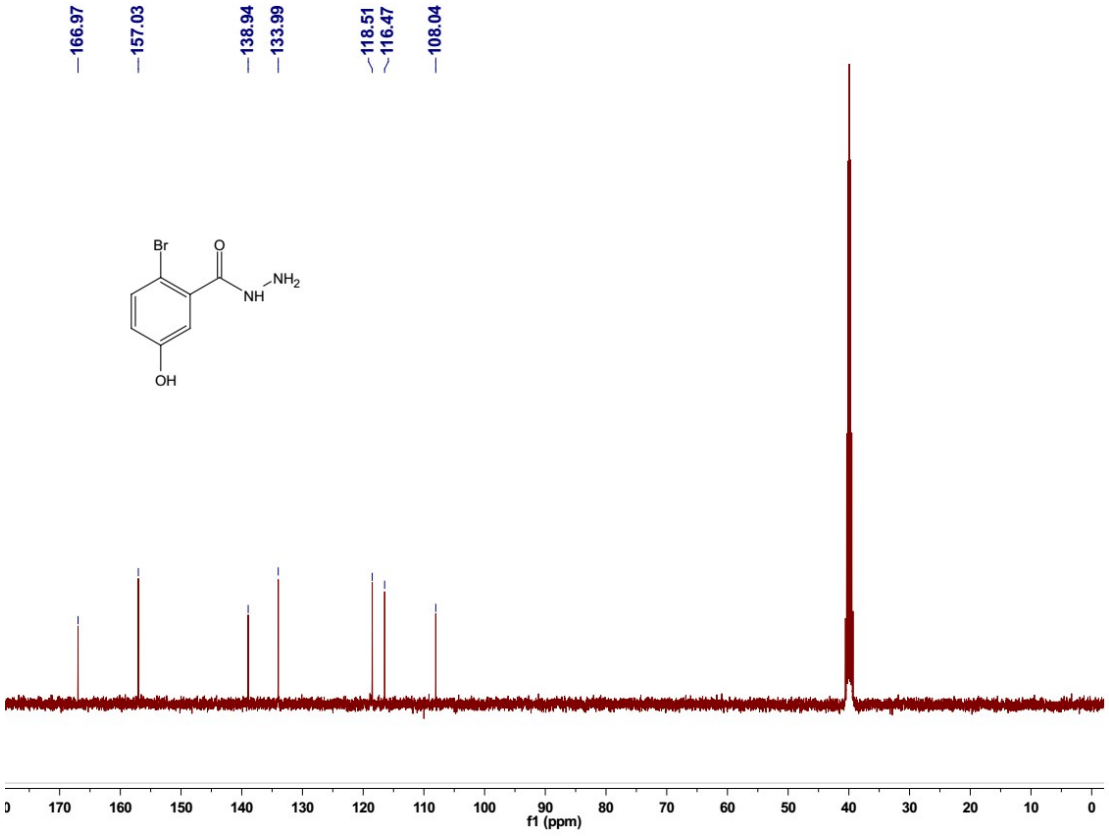



**Figure S18.** $^{13}$C NMR spectrum of **2b** in DMSO-$d_6$ solution

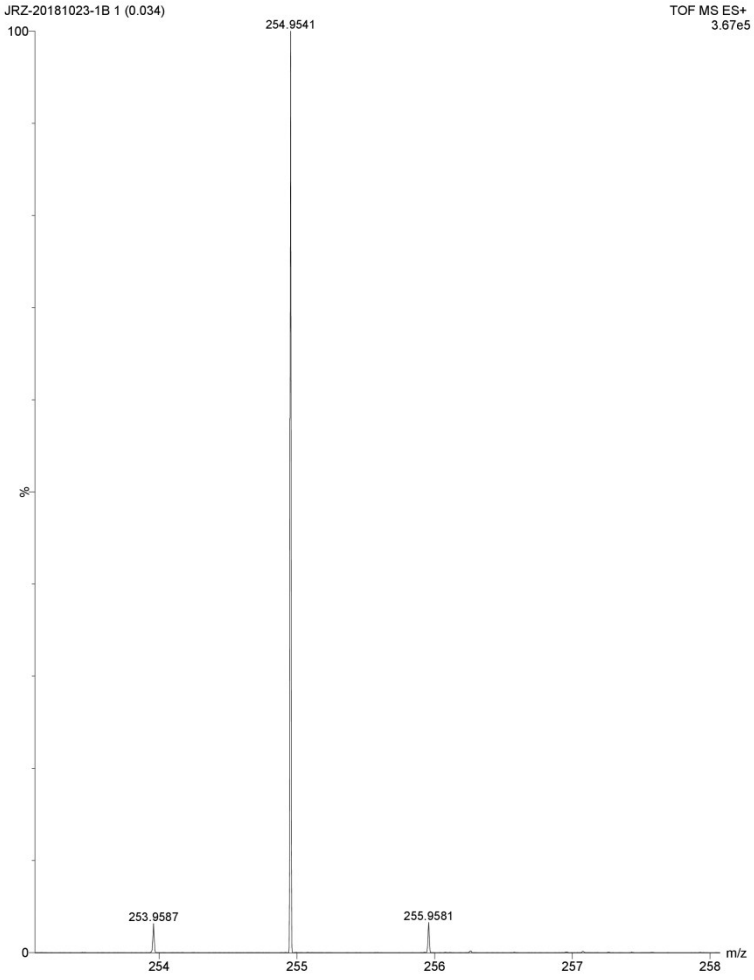



**Figure S19.** High resolution mass spectrum of **2b**.

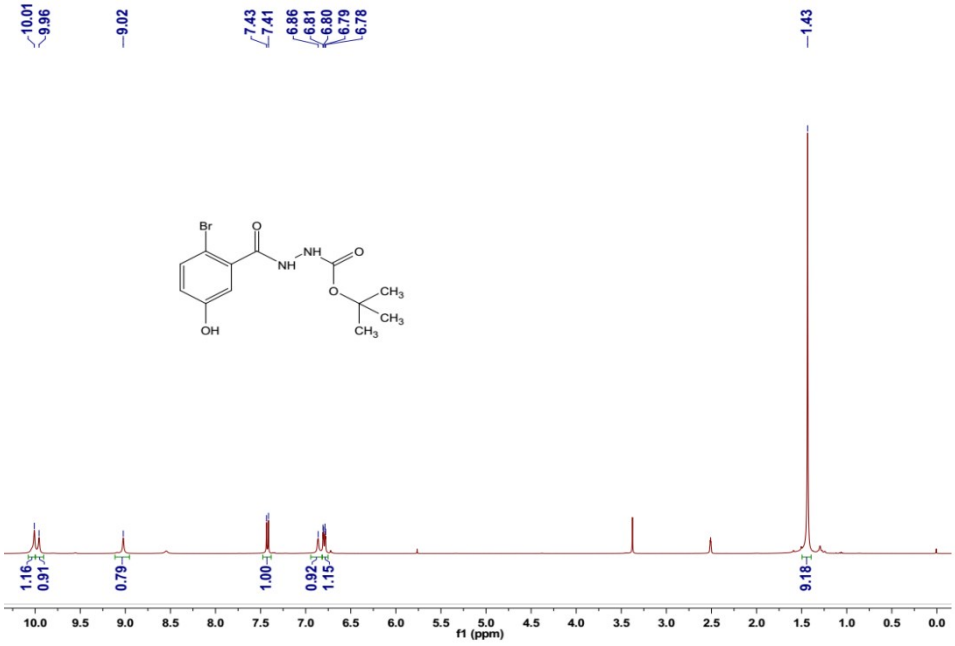



**Figure S20.** $^{1}$H NMR spectrum of **2c** in DMSO-$d_6$ solution.

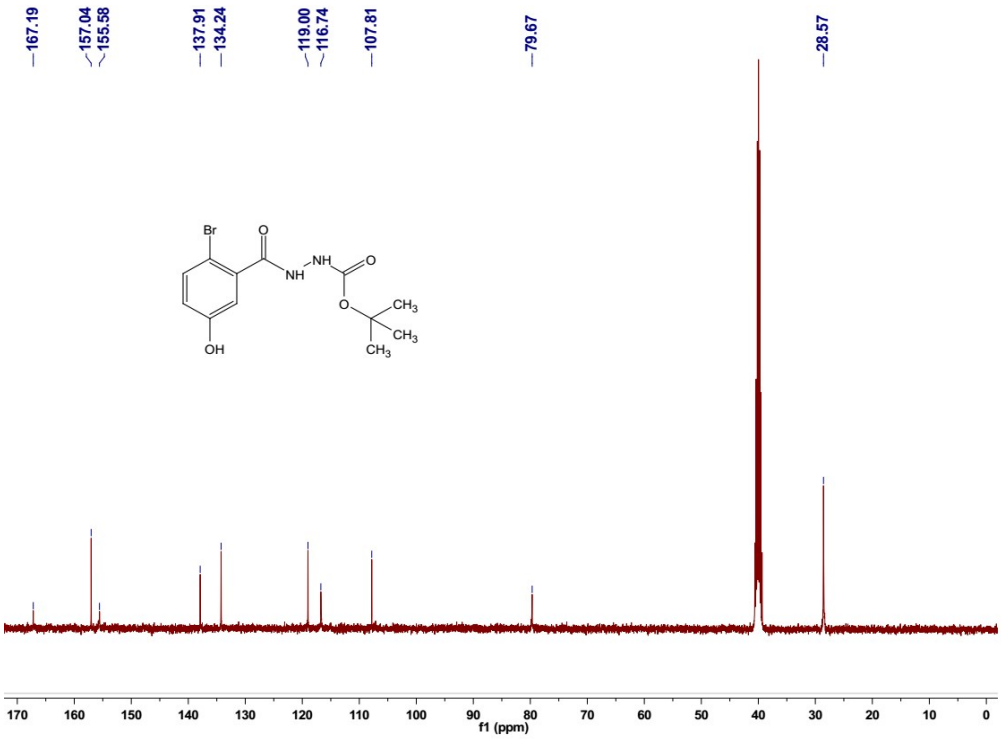



**Figure S21.** $^{13}$C NMR spectrum of **2c** in DMSO-$d_6$ solution

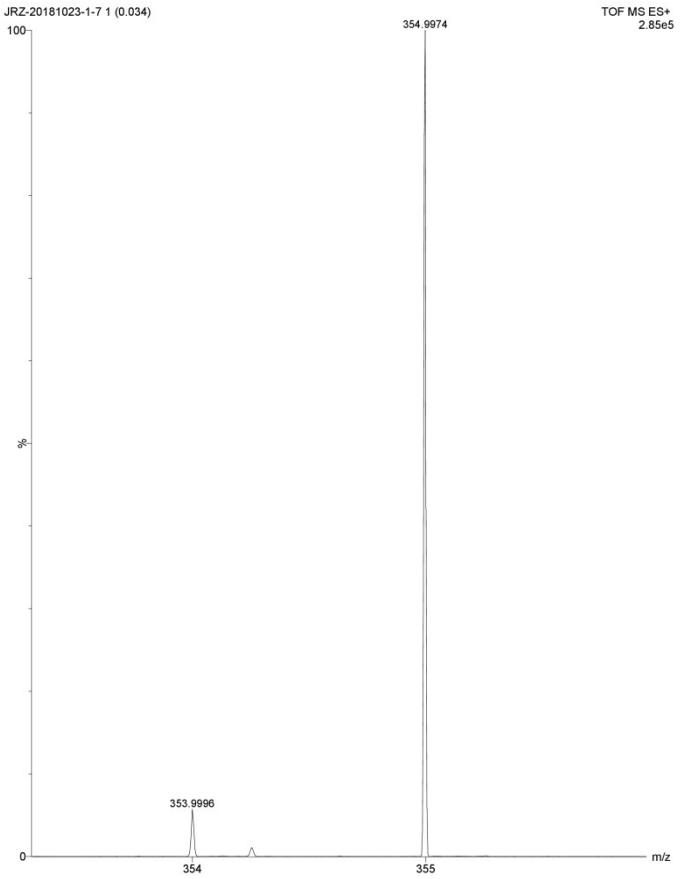



**Figure S22.** High resolution mass spectrum of **2c**.

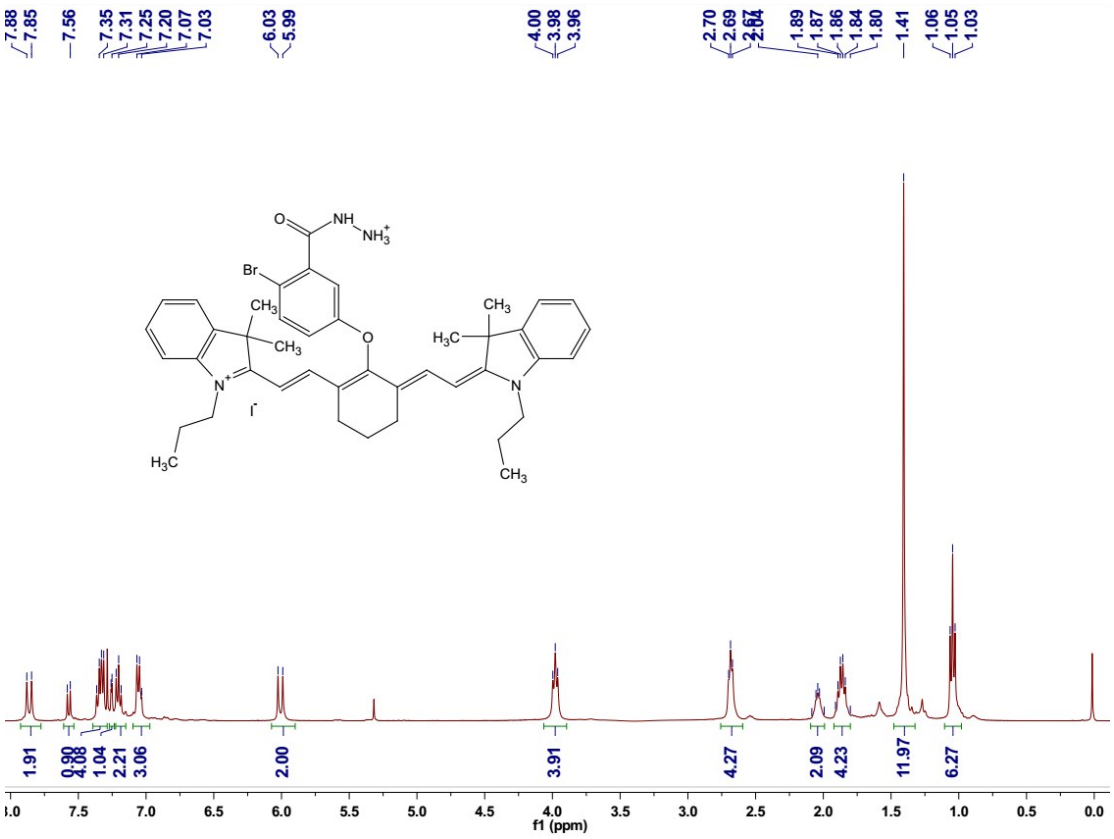



**Figure S23.** $^{1}$H NMR spectrum of **compound 2 (AlkaP-1)** in $CDCl_3$ solution.

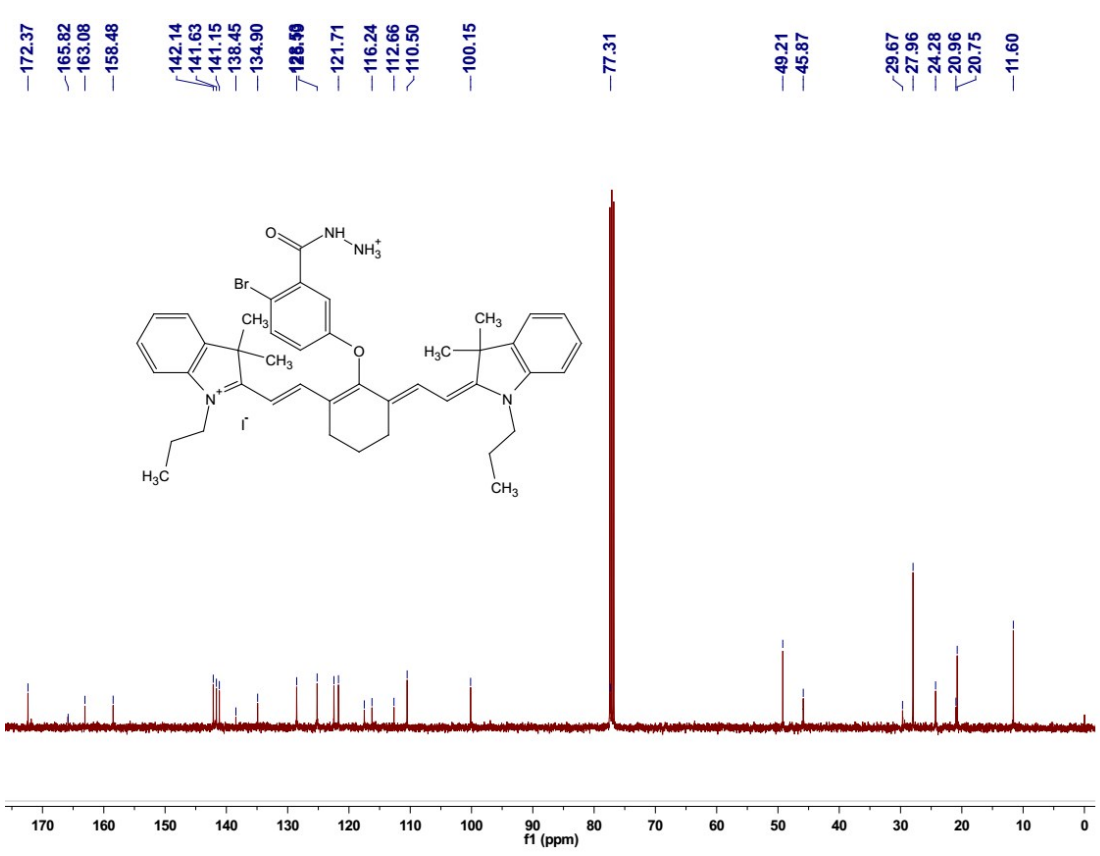



**Figure S24.** $^{13}$C NMR spectrum of **compound 2 (AlkaP-1)** in $CDCl_3$ solution

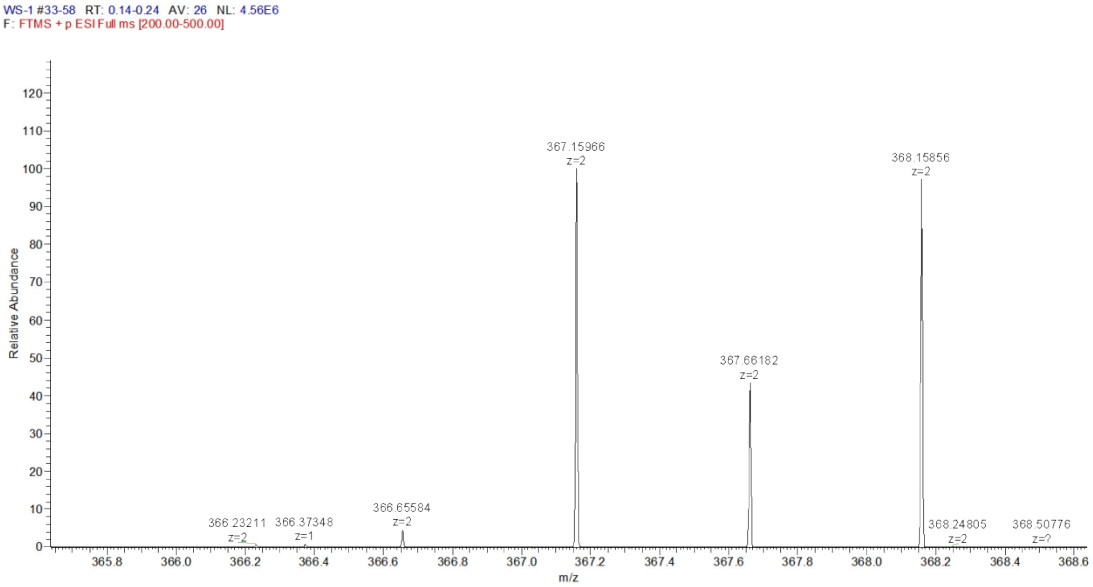



**Figure S25.** High resolution mass spectrometry of **compound 2 (AlkaP-1)**.

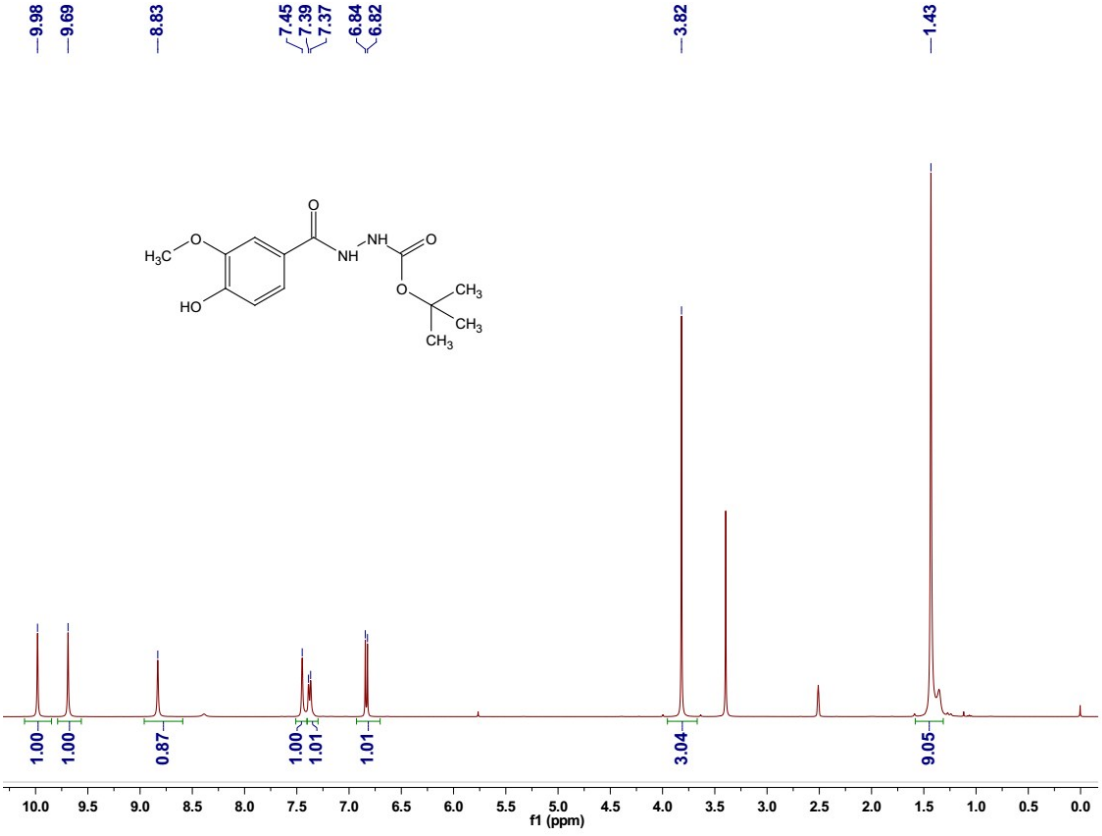



**Figure S26.** $^{1}$H NMR spectrum of **3b** in DMSO-$d_6$ solution.

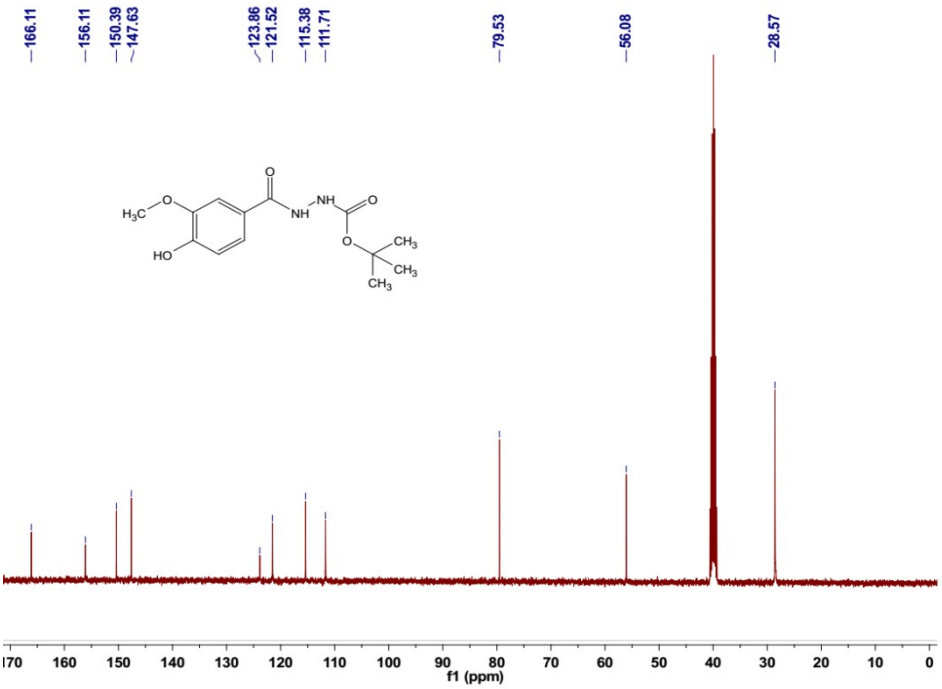



**Figure S27.** $^{13}$C NMR spectrum of **3b** in DMSO-$d_6$ solution

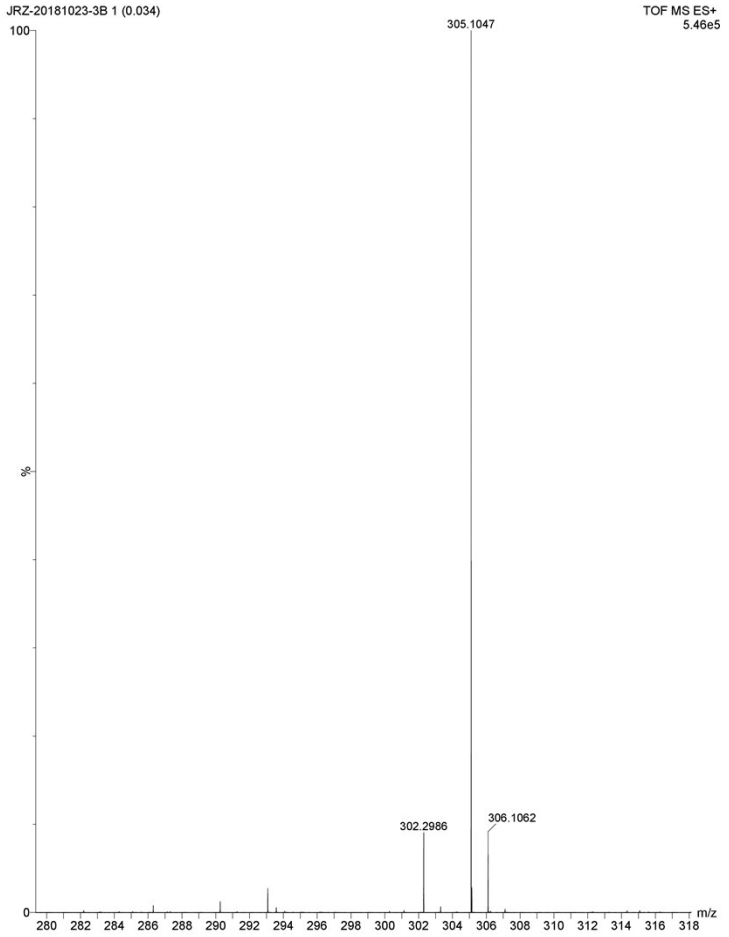



**Figure S28.** High resolution mass spectrum of **3b**.

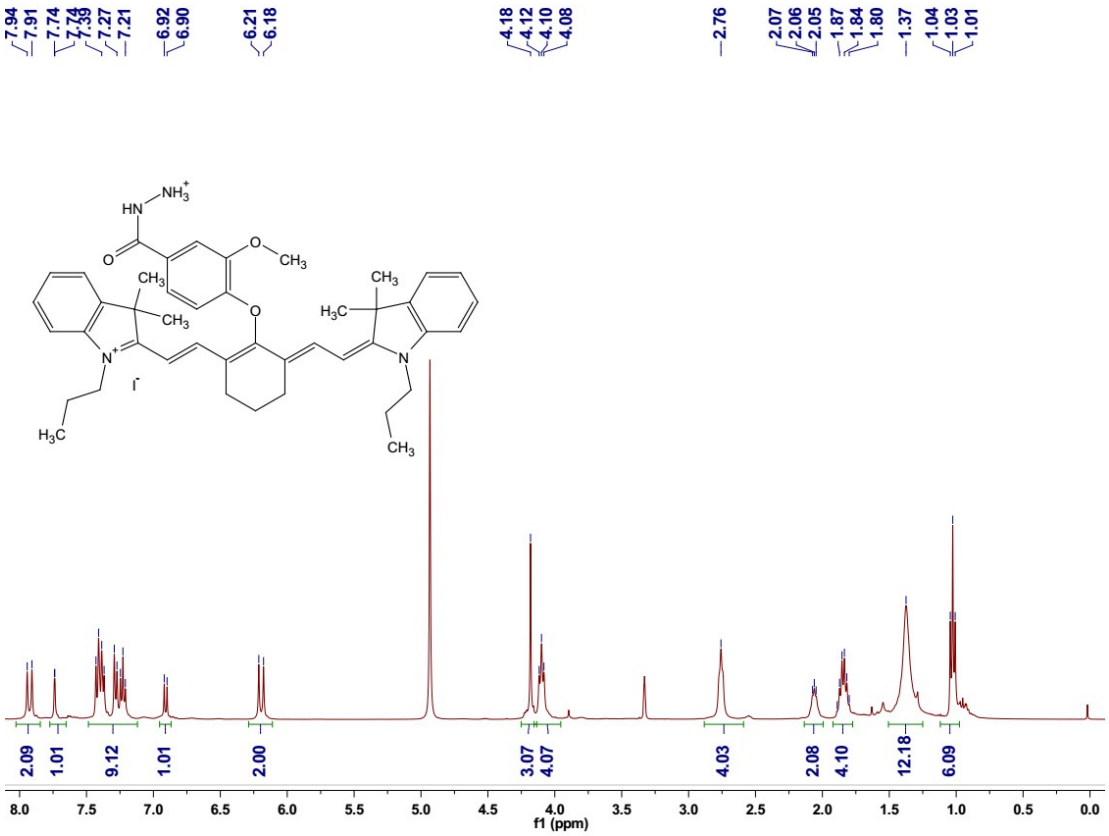



**Figure S29.** $^{1}H$ NMR spectrum of **compound 3** in $CD_3OD$ solution.

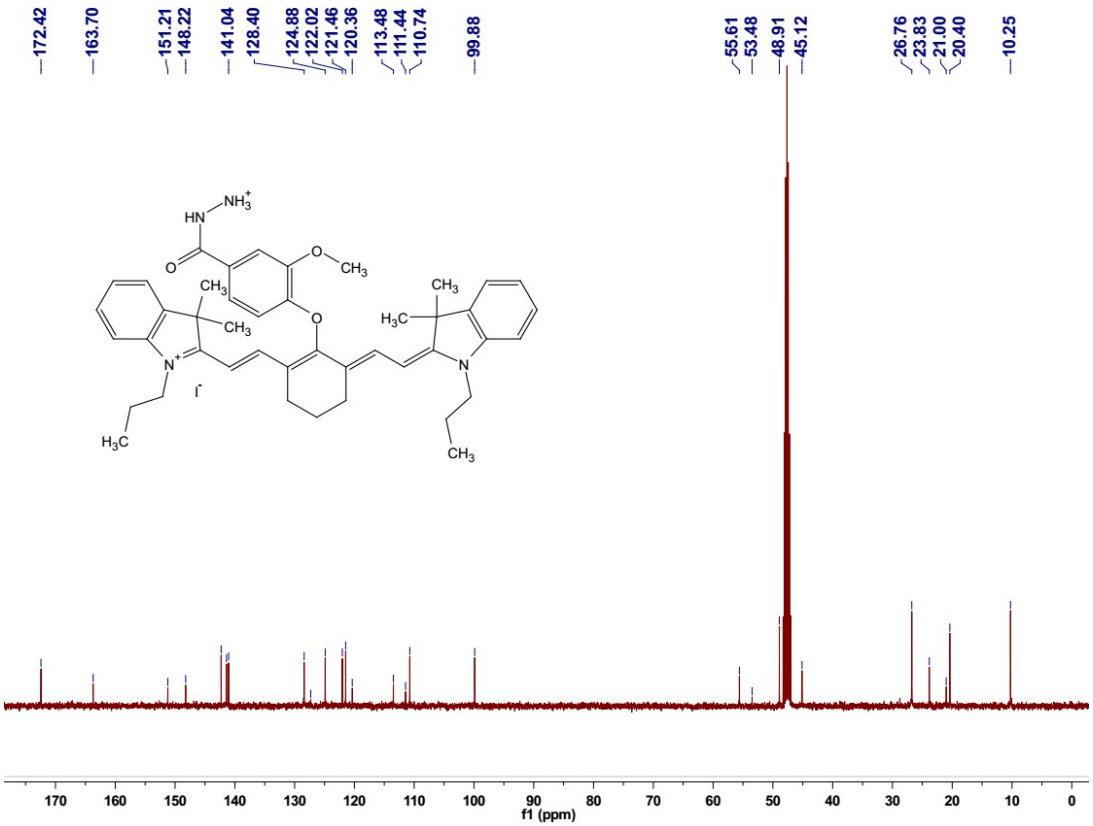



**Figure S30.** $^{13}C$ NMR spectrum of **compound 3** in $CD_3OD$ solution

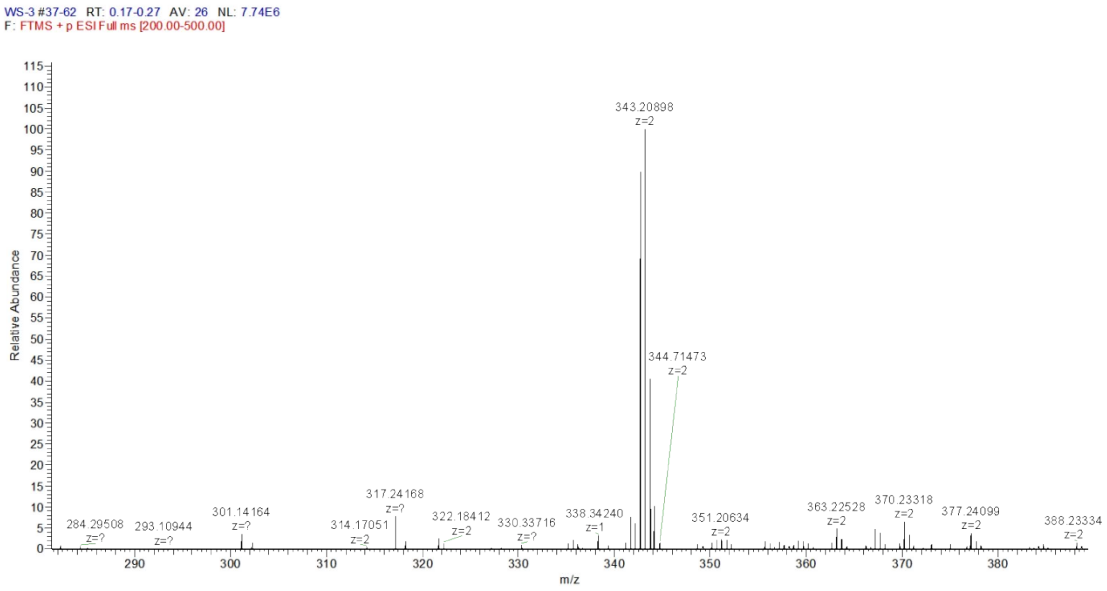



**Figure S31.** High resolution mass spectrum of **compound 3**.

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