Supporting Information for

A near-infrared fluorogenic probe for nuclear thiophenol detection

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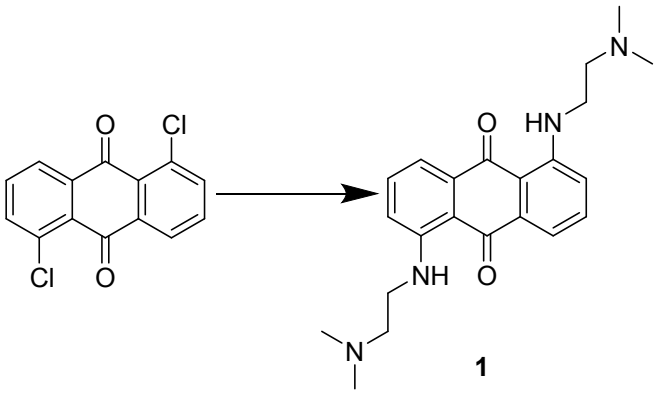
Table of contents

1. Materials and instruments

Unless otherwise stated, all reagents for synthesis were purchased from commercial suppliers and were used without further purification. 1,5-dichloroanthraquinone was purchased from Bide Pharmatech. Ltd. (Shanghai, China). 2,4-dinitrofluorobenzene was bought form Macklin Biochemical Co., Ltd (Shanghai, China). The wild type zebrafish (5 dpf) was obtained from Nanjing EzeRinka Biotechnology Co., Ltd. The nude mice (20 g) were purchased from Jinan Shengwei Biotechnology Co., Ltd. The probe AQD was dissolved in DMSO to make 1.0 mM stock solution.

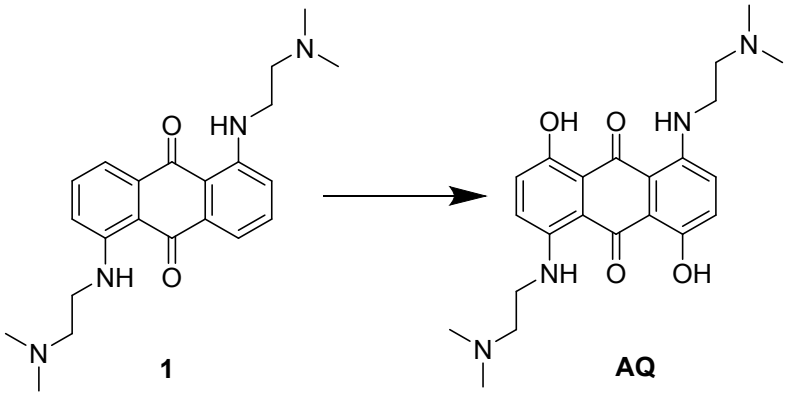
pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China). Absorption spectra were recorded on a UV-1700 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were obtained with an F-4600 fluorescence spectrometer (Hitachi High-Tech Science Co., Ltd., Japanese). ^{1}H NMR and ^{13}C NMR spectra were taken on a 400 MHz spectrometer (Bruker Co., Ltd., Germany), δ values are in ppm relative to TMS, coupling constants (*J* values) are reported in hertz. HRMS spectra were obtained on a maxis ultra-high resolution-TOF MS system (Bruker Co., Ltd., Germany). MTT assay was performed using a TRITURUS microplate reader. The confocal fluorescence images were taken using TCS SP8 confocal laser scanning microscope (Leica Co., Ltd., Germany). The fluorescent imaging of mice was performed on IVIS Lumina III system (Xenogen, USA) with a metal halide lamp (150 W).

2. Synthetic procedures and characterization details[1-3]



1

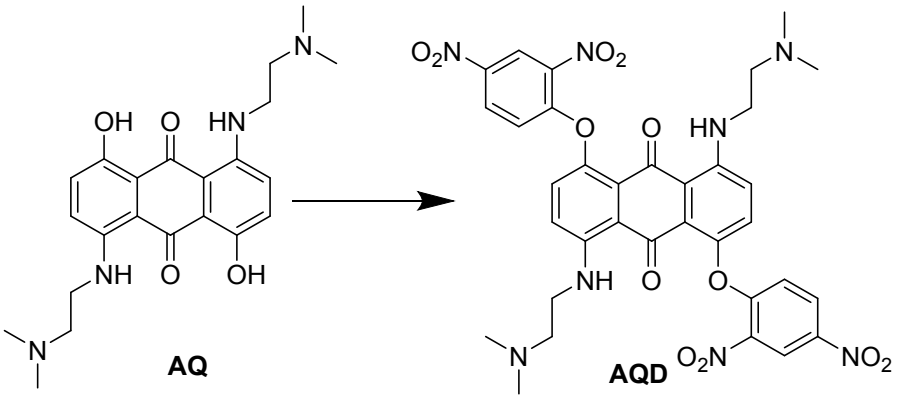
The mixture of 1,5-dichloroanthraquinone (7.5 g, 27 mmol) in *N,N*-dimethylethylenediamine (23.8 g, 270 mmol) was heated and refluxed for 18 h. After that, the reaction solution was cooled to room temperature and diluted with water to precipitate out the crude product. The suspension was filtered and the solid was recrystallized from methanol to afford compound **1** as a purple solid (7.5 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (t, J = 4 Hz, 2H), 7.57 (d, J = 8 Hz, 2H), 7.51 (t, J = 8 Hz, 2H), 6.96 (d, J = 8 Hz, 2H), 3.42 (dd, J = 12, 4 Hz, 4H), 2.68 (t, J = 6.4 Hz, 4H), 2.36 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.40, 151.17, 136.29, 135.16, 116.29, 114.93, 113.16, 58.06, 45.57, 40.95. HRMS-ESI (m/z): calculated for $C_{22}H_{28}N_4O_2$ $[M+H]^+$ 381.2285, found 381.2267.



1 **AQ**

The mixture of compound **1** (6 g, 15.73 mmol) in concentrated sulfuric acid (65 g) was cooled to –10 °C. Anhydrous sodium chlorate (6.5 g, 61.6 mmol) was added in portions over 1.5 h. Then the mixture was stirred for 3 h at room temperature. After that, the cold cold sodium hydrogen sulfite solution (1%, 1000 mL) was added slowly

to the reaction solution followed by alkalized to pH = 7 with 5 M sodium hydroxide. The resulting solution was extracted with CH_2Cl_2, concentrated, and further purified by column chromatography on silica gel (CH_2Cl_2/ MeOH = 9:1, v/v) to afford compound **AQ** as a blue solid (1.0 g, 16.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.97 (s, 2H), 9.77 (t, *J* = 4 Hz, 2H), 7.165 (d, *J* = 12 Hz, 2H), 7.015(d, *J* = 12 Hz, 2H), 3.42 (dd, *J* = 12, 4 Hz, 4H), 2.66 (t, *J* = 6.4 Hz, 4H), 2.35 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 186.50, 155.07, 146.93, 128.67, 120.74, 115.11, 109.52, 58.23, 45.65, 41.16. HRMS-ESI (m/z): calculated for $C_{22}H_{28}N_4O_2$ $[M+H]^+$ 413.2183, found 413.2160.



To a mixture of compound **AQ** (200 mg, 0.49 mmol) in 10 mL of dry *N,N*-dimethylformamide was added sodium hydride (60% dispersion in mineral oil, 60 mg, 1.5 mmol) and then the mixture was stirred at 40 °C for 1.5 h. After that, 2,4-dinitrofluorobenzene (280 mg, 1.5 mmol) was added to the reaction solution, and the mixture was heated to 80 °C for another 2 h. The crude product further was purified through silica gel column chromatography (CH_2Cl_2/ MeOH = 9:1, v/v) to afford compound **AQD** as a purple solid (109 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.56 (s, 2H), 8.94 (s, 2H), 8.23 (d, *J* = 8 Hz, 2H), 7.34 (d, *J* = 8 Hz, 2H), 7.04 (d, *J* = 8 Hz, 2H), 6.86 (d, *J* = 8 Hz, 2H), 3.33 (d, *J* = 4 Hz, 4H), 2.59 (t, *J* = 6.4 Hz, 4H), 2.29 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 183.44, 158.04, 149.61, 140.80, 140.45, 138.88, 131.86, 128.62, 125.78, 122.07, 118.55, 116.43, 113.02, 57.49, 45.35, 41.02. HRMS-ESI (m/z): calculated for $C_{22}H_{28}N_4O_2$ $[M+H]^+$ 745.2212, found 745.2193.

3. Selectivity assay

For the selectivity experiment, the stocks solutions of the reactive species were prepared in sartorius ultrapure and used immediately. Additionally, prior to reaction, all the solutions for air-sensitive reducing agents (thiophenol, Hcy, Cys, GSH, H_2S, Vc, HSO_3^-, and Fe^{2+}) were degassed with N_2. Preparation details for reactive species and metal ions are described as follows:

The concentration of H_2O_2 was determined from the absorption at 240 nm (ε= 43.6 $M^{-1}cm^{-1}$). Hydroxyl radical (•OH) was produced by Fenton reaction. To generate •OH, Fe^{2+} solution was added in the presence of 10 equiv of H_2O_2. The concentration of •OH was equal to the Fe^{2+} concentration. Singlet oxygen ($^1O_2$) was produced by the reaction of H_2O_2 with ClO^-. The concentration of ClO^- was determined from the absorption at 292 nm (ε= 350 $M^{-1}cm^{-1}$). Superoxide solution ($O_2^{\bullet-}$) was prepared by adding KO_2 to dry DMSO and stirring vigorously for 30 min. Peroxynitrite ($ONOO^-$) solution was synthesized according to literature,[4] and the concentration of $ONOO^-$ was determined from the absorption at 302 nm (1670 $M^{-1}cm^{-1}$). NO was generated by SNP (Sodium Nitroferricyanide (III) Dihydrate). Hydrogen sulfide (H_2S) was prepared from NAHS solid. In addition to aforementioned species, the rest of reactive species (HCy, Cys, GSH, Vc) and metal ions (Na^+, K^+, Cd^{2+}, Pd^{2+}, Co^{2+}, Mg^{2+}, Hg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+}) were prepared by dissolving corresponding solid into sartorius ultrapure water.

4. The MTT assay

The MTT assay was conducted to evaluate the cytotoxicity of **AQD**. Hela cells were replanted in the 96-well micro plates to a total volume of 200 μL $well^{-1}$. The plates were maintained at 37°C, 5% CO_2/95% air incubator for 24 hours. Then, the cells were incubated with different concentrations **AQD** (0, 10, 30, 50, 70, 90 μM) for another 8 hours. Subsequently, the culture medium was removed and MTT solution

(5.0 mg ml^{-1}) was added to each well. After 4 hours, the remaining MTT solution was removed and 150 μL DMSO was added to dissolve the formazan crystals. The absorbance of solution was measured at 490 nm with 5 min gentle agitation using a TRITURUS microplate reader.

5. Confocal fluorescent imaging in living cells

The Hela cells were seeded into 18 mm glass-bottom dishes with high-glucose DMEM medium (10% fetal bovine serum, 1% penicillin, and 1% streptomycin) and maintained in incubator (37°C, 5% CO_2/95% air) for 24 h. For time-dependent imaging assays, the cells were firstly incubated with thiophenol (10 μM) for 15 min, and then washed with PBS buffer for three times. After that, the cells were stained with **AQD** (10 μM) and imaged immediately. For thiophenol dose-dependent imaging assays, the cells were treated with different concentrations of thiophenol (0, 5, 10 μM) for 15 min and washed with PBS buffer for three times, and then incubated with **AQD** (10 μM) for 60 min before imaged. For the nuclear-targeted imaging assays, Hela cells were treated with thiophenol (10 μM) for 15 min and washed with PBS buffer for three times. After that, the cells were stained with **AQD** (10 μM) for 60 min followed by co-incubated with DAPI (10 μg/mL) for 15 min. Then, the cells were washed with PBS buffer for three times and imaged. DAPI channel was imaged with λex = 405 nm and emission collection in 430−480 nm. **AQD** channel was obtained with λex = 633 nm and emission collection in 640−730 nm.

6. Confocal fluorescent imaging in zebrafish

The zebrafish experiments were in agreement with the guidelines of the Institutional Animal Care and Use Committee. The wild-type zebrafish were soaked with various concentrations of thiophenol (0, 5, 10 μM) for 15 min, and then washed with PBS for three times. After that, the zebrafish were incubated with **AQD** (10 μM) for 60 min

followed by anesthetized with tricaine (1.0 μg/mL) for 10 min and then imaged. The fluorescent images were recorded with 633 nm excitation and 640−730 nm collection.

7. Fluorescent imaging in mice

The nude mice (~20 g) were fasted for 12 h to avoid the possible fluorescence interference. PBS buffer solution (200 μL) containing various concentrations of thiophenol (0, 20, 40 μM) was injected intraperitoneally. After 15 min, PBS buffer solution (200 μL) containing **AQD** (40 μM) was injected intraperitoneally. Before imaging, the mice were anesthetized with 4% chloral hydrate (15 mL/kg) by intraperitoneal injection. The images were acquired with excitation filter 600 nm and emission filter 670 nm.

Reference

1. K. C. Murdock, R. G. Child, P. F. Fabio, R. D. Angier, R. E. Wallace, F. E. Durr and R. V. Citarella, *J. Med. Chem.*, 1979, **22**, 1024-1030.
2. A. P. Krapcho, Z. Getahun, K. L. Avery, K. J. Vargas, M. P. Hacker, S. Spinelli, G. Pezzoni and C. Manzotti, *J. Med. Chem.*, 1991, **34**, 2373-2380.
3. J. S. Al-Otaibi and T. M. El Gogary, *J. Mol. Struct.*, 2017, **1130**, 799-809.
4. T. Peng, N.-K. Wong, X. Chen, Y.-K. Chan, Z. Sun, J. J. Hu, J. Shen, H. El-Nezami and D. Yang, *J. Am. Chem. Soc.*, 2018, **140**, 854-854.

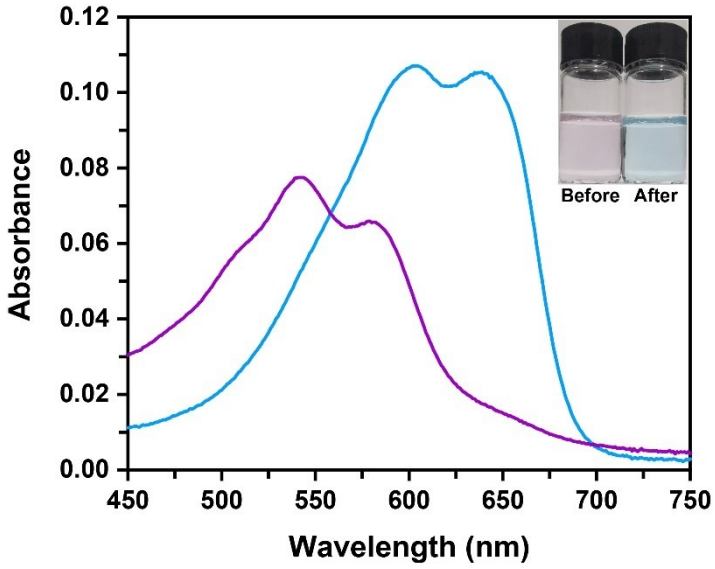


Fig. S1 Absorption spectra of **AQD** (10 μM) in the absence (purple line) or presence (blue line) of thiophenol (10 μM). Inset: the color changes of **AQD** solution before (light purple) and after (light blue) thiophenol addition. The results were recorded after incubation of **AQD** with thiophenol for 60 min in HEPES buffer (20 mM, pH 7.4, 30%DMSO) at 37 °C.

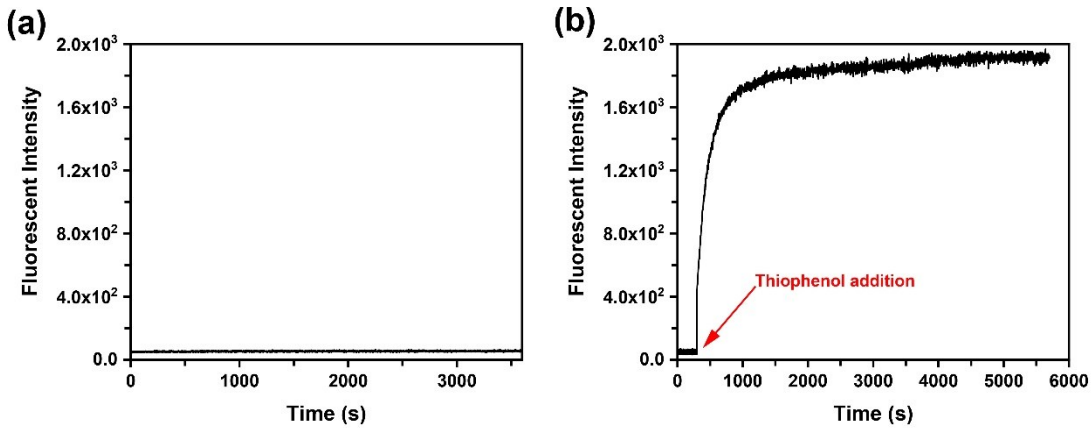


Fig. S2. Time-dependent fluorescence intensity change of **AQD** (10 μM) without (a) or with (b) thiophenol (10 μM) in HEPES buffer (20 mM, pH 7.4, 30%DMSO). λex/λem = 600/680 nm. Slit width: 5 nm/5 nm.

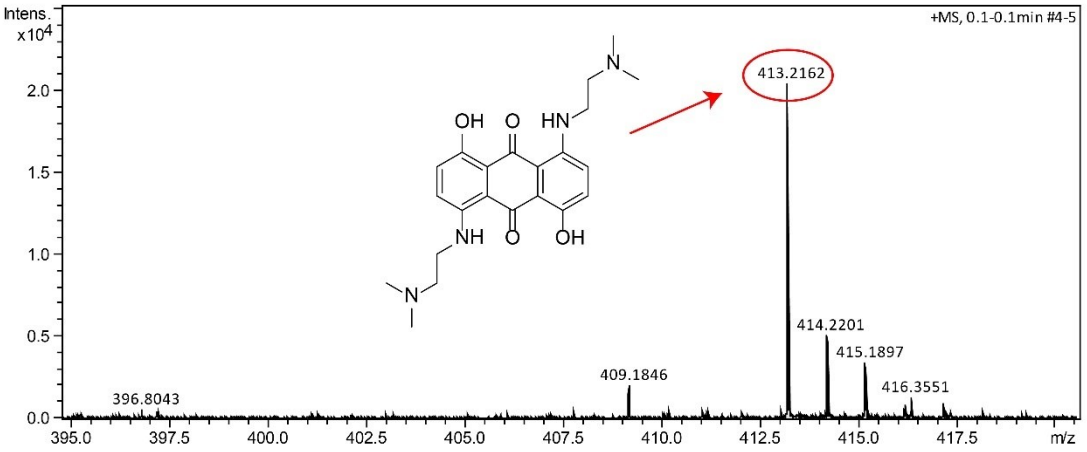


Fig. S3. HRMS spectral analysis of reaction between **AQD** and thiophenol.

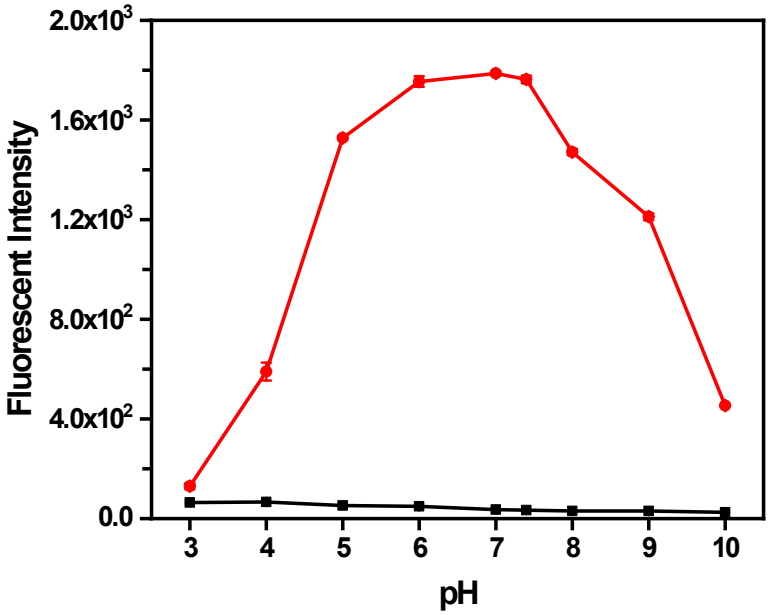


Fig. S4 Fluorescence intensity change of **AQD** (10 μM) with (red line) or without (black line) thiophenol (10 μM) under different pH values (3.0, 4.0, 5.0, 6.0, 7.0, 7.4, 8.0, 9.0, 10.0) by using universal buffer solution (0.1 mM citric acid, 0.1 M KH_2PO4, 0.1 M $Na_2B_4O_7$, 0.1 M Tris, 0.1 M KCl) with 30% DMSO. λex/λem = 600/680 nm. Slit width: 5 nm/5 nm.

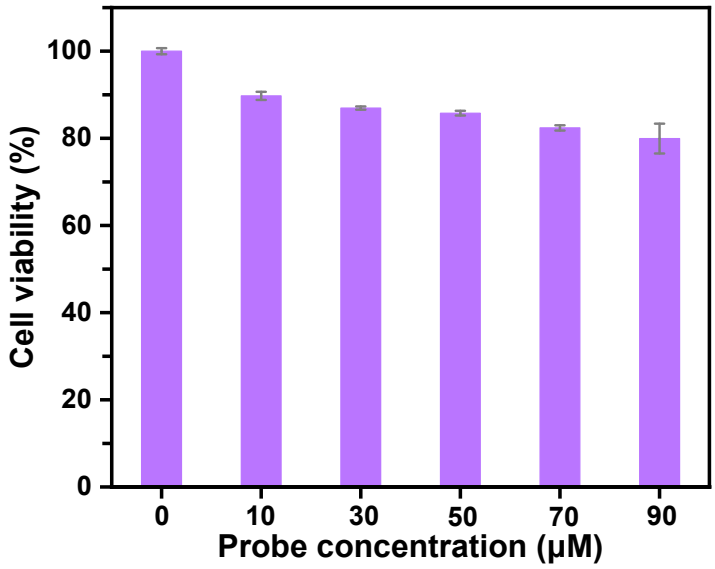


Fig. S5 MTT assay of Hela cells with different concentrations of **AQD**. The IC50 value was calculated to be 207 μM.

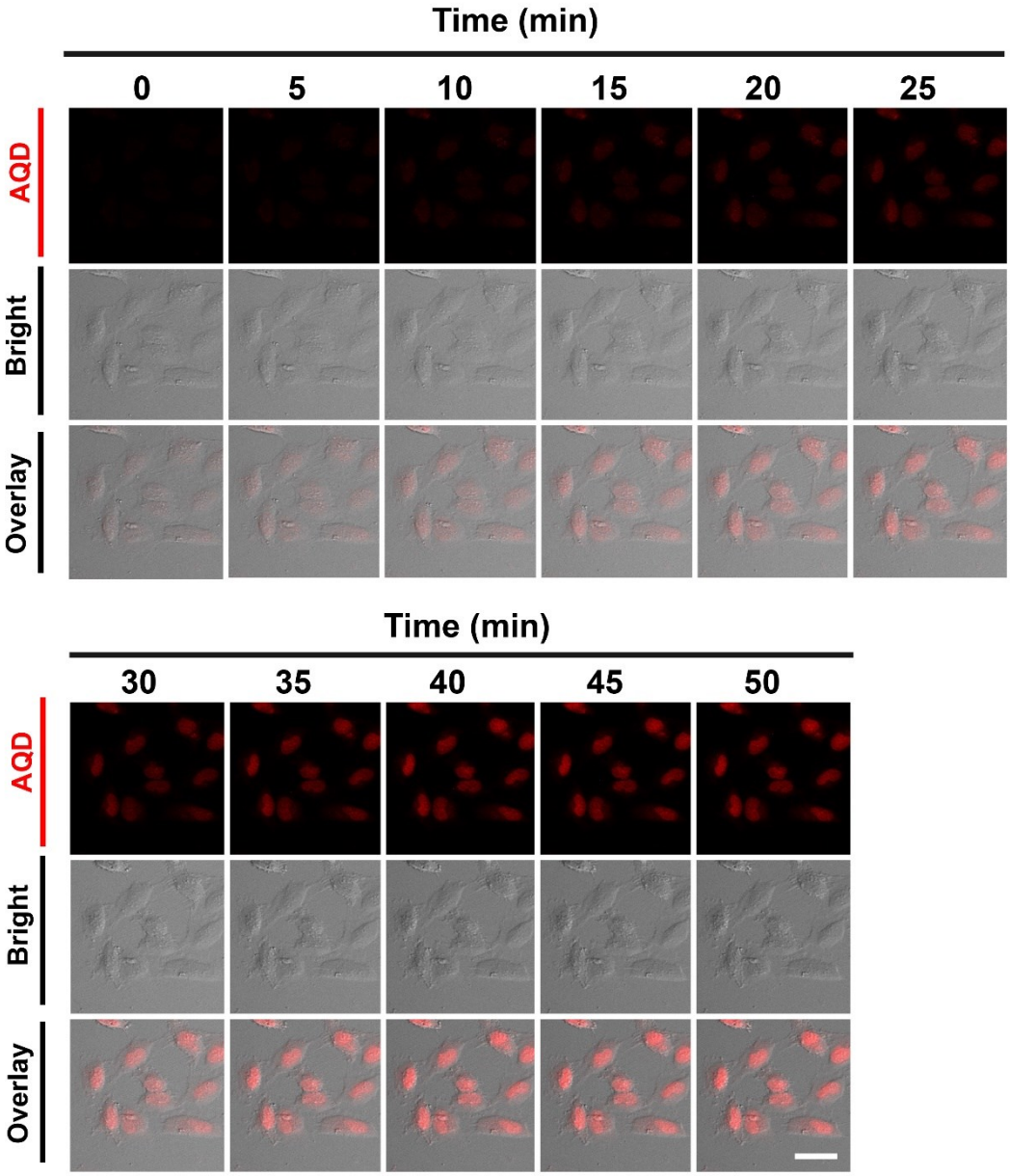


Fig. S6 Time-dependent fluorescence imaging of **AQD** in Hela cells. The cells were pretreated with thiophenol (10 μM) for 15 min and then incubated with **AQD** (10 μM) followed by imaged at different time points (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 min). The images were recorded with 633 nm excitation and 640−730 nm collection. Scale bar = 20 μm.

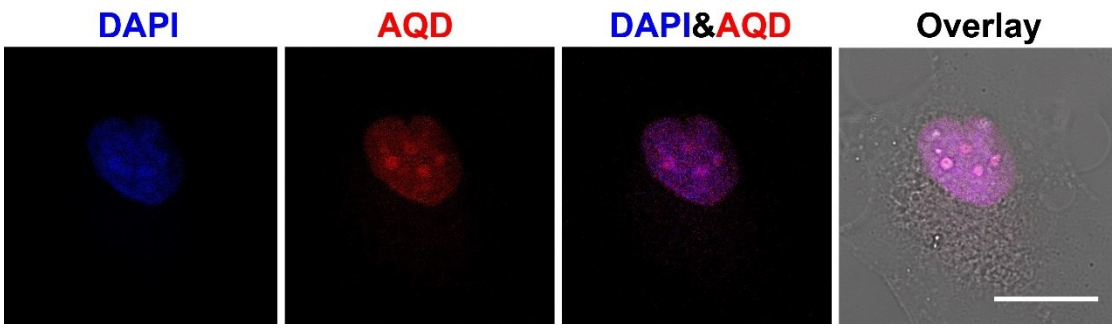


Fig. S7 Nuclear targeting investigation of **AQD**. Hela cells were treated with thiophenol (10 μM, 15 min) and then stained with **AQD** (10 μM, 60 min) followed by incubated with DAPI (10 μg/mL, 15 min). DAPI was imaged in blue channel with λex = 405 nm and emission collection in 430−480 nm. **AQD** was obtained in red channel with λex = 633 nm and emission collection in 640−730 nm. Scale bar = 20 μm.

7. NMR spectra

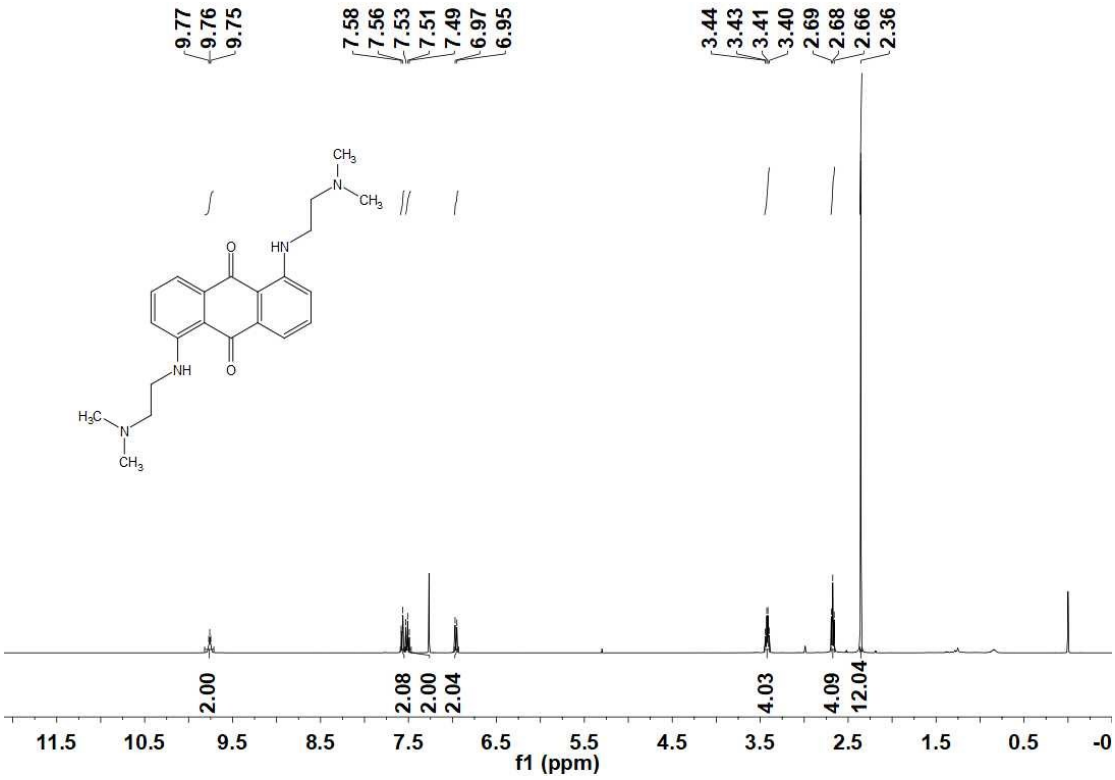


Fig. S8 ^{1}H NMR spectra of compound **1** ($CDCl_3$).

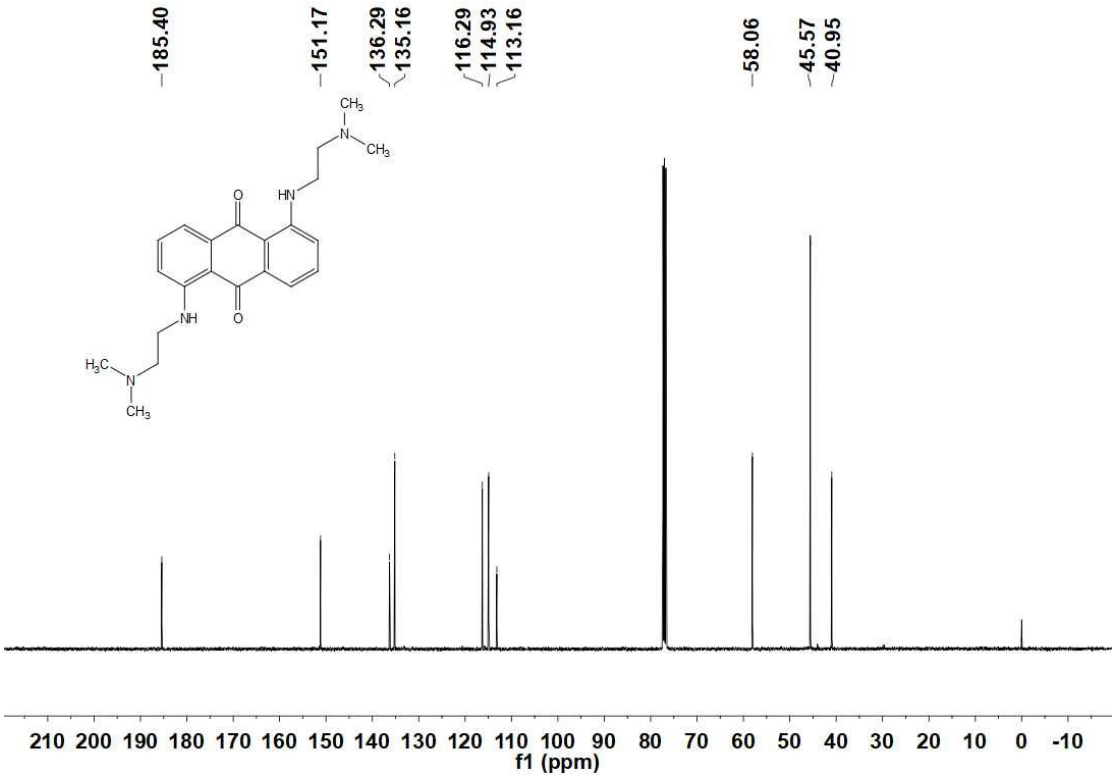


Fig. S9 ^{13}C NMR spectra of compound **1** ($CDCl_3$).

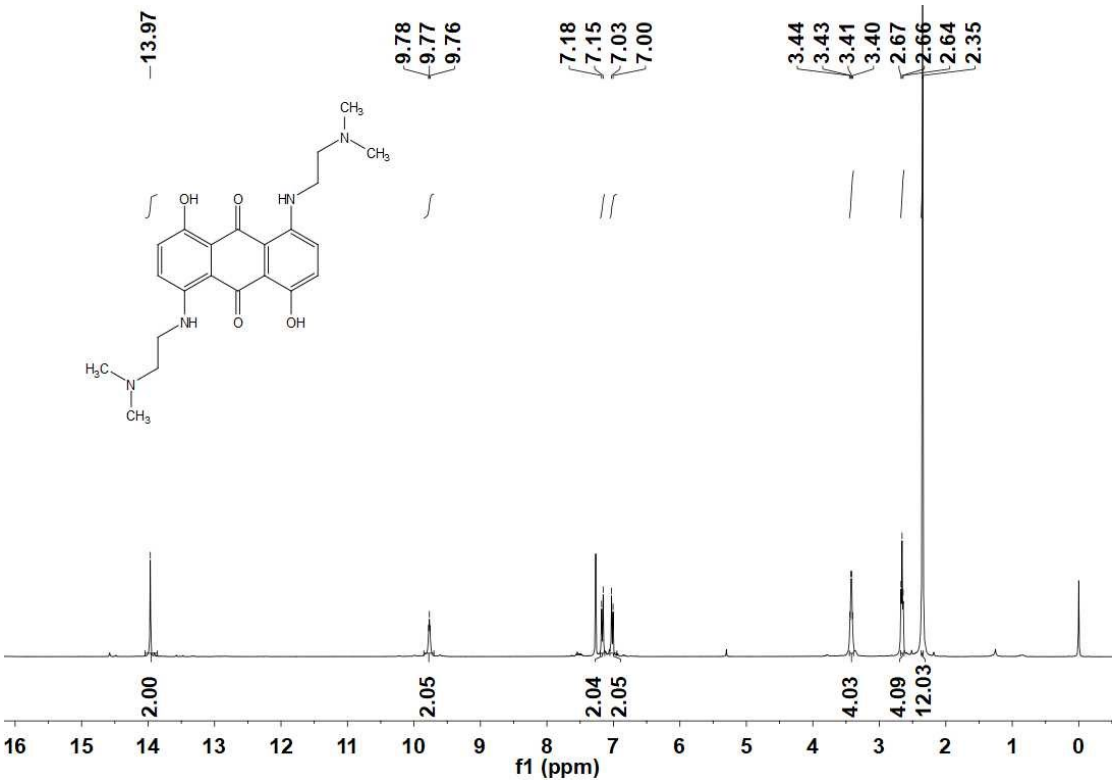


Fig. S10 ^{1}H NMR spectra of compound **AQ** ($CDCl_3$).

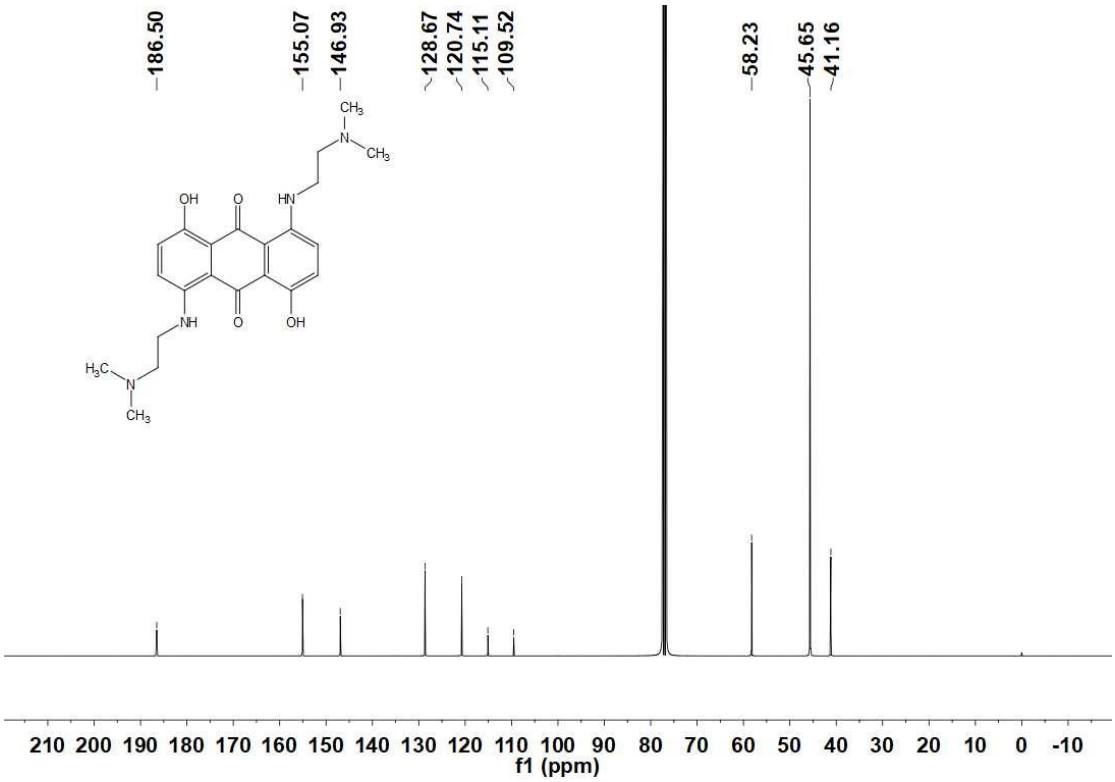


Fig. S11 ^{13}C NMR spectra of compound **AQ** ($CDCl_3$).

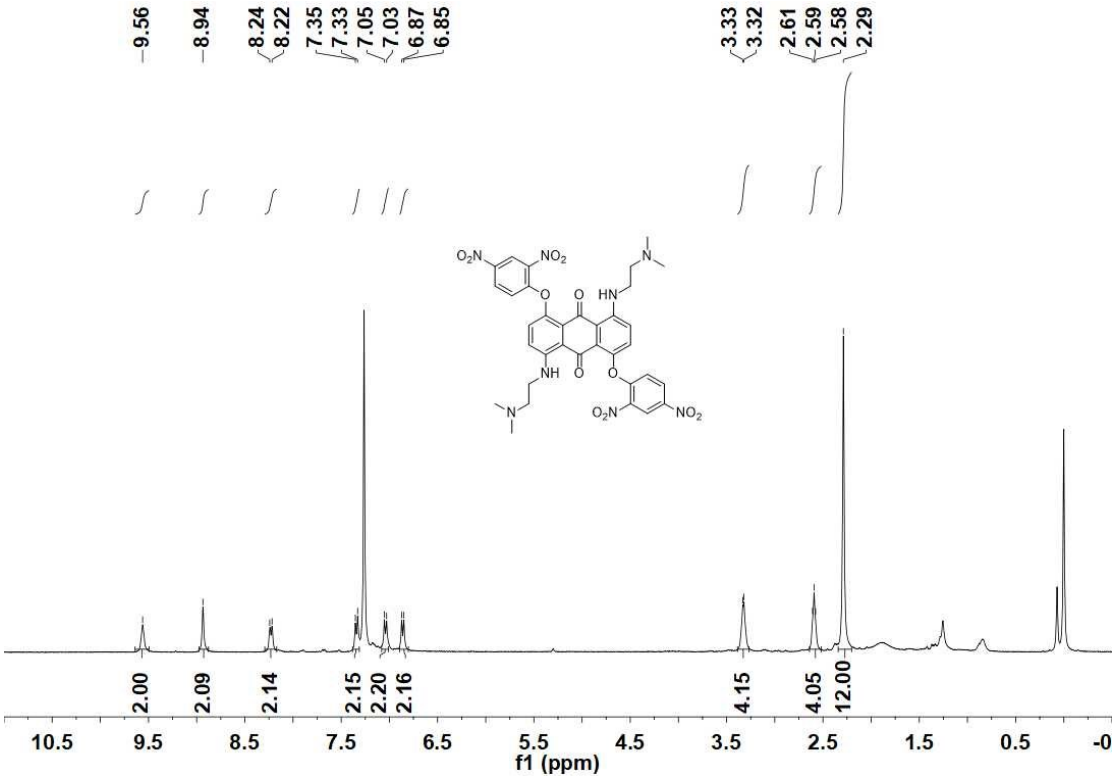


Fig. S12 ^{1}H NMR spectra of compound **AQD** ($CDCl_3$).

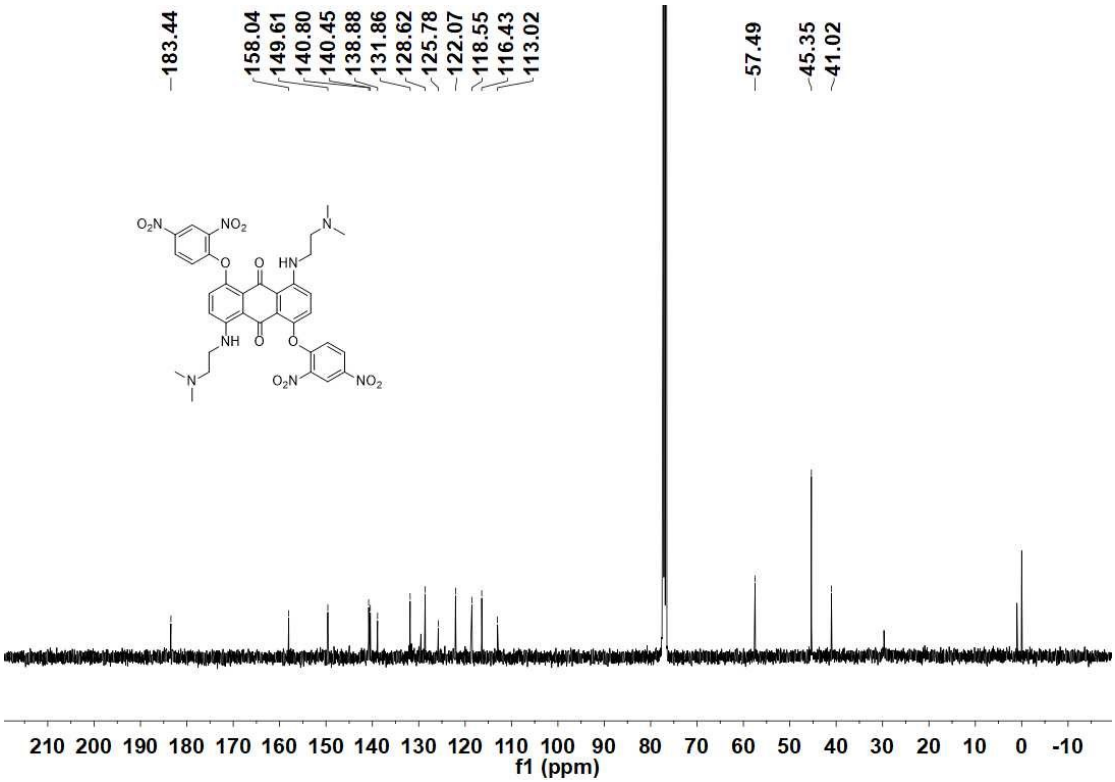


Fig. S13 ^{13}C NMR spectra of compound **AQD** ($CDCl_3$).