Supporting Information for

Real-time visualization of sulfatase in living cells and in vivo with a ratiometric AIE fluorescent probe

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Experimental section

Main Materials

All chemical reagents and solvents were purchased from qualified commercial suppliers and used directly. 5-Bromothiophen-2-formaldehyde, *p*-hydroxy phenylboronic acid, tetri (triphenylphosphine) palladium, potassium carbonate, tetrahydrofuran, methanol, piperidine, dimethyl sulfoxide (DMSO) and dimethyl sulfoxide deuterium were purchased from Shanghai Maclin Biochemical Technology Co. LTD. Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium, phosphoric acid buffer salt solution (PBS) are purchased from Jiangsu Kaiji Biotechnology Co., LTD. Fetal bovine Serum (FBS), 3-(4, 5-dimethyl-2-thiazole)-2, 5-diphenyl tetrazolium bromide (MTT), trypsin were purchased from Thermo Fisher Scientific. Sulfatase from Helix pomatia S9626 was obtained from Sigma-Aldrich. All reagents are purchased from commercial sources and used and stored according to the specifications in the instructions.

Instruments

^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Advance-300 and Advance-500 spectrometer (Bruker, Germany) using Deuterium DMSO as solvent. Mass spectra were recorded using an ABI Mariner ESI-TOF spectrometer (Applied Biosystems, Thermo Fisher Scientific, USA). Absorption spectra were measured on a UV-vis spectrophotometer (One-drop, Nanjing, China). Steady-state fluorescence spectra were recorded on 1-cm quartz cells using Fluorescence Spectrometers F-7100 (Hitachi). The cell viability was detected by the MTT kit, and the absorbance of each sample in a 96-well plate was measured at 490 nm using a microplate reader (BioTek). The cellular fluorescence images were taken by the inverted fluorescence microscope (Leica, DMi8, Germany) and confocal laser scanning microscope (FluoView™, FV1000, Olympus, Japan). An IVIS Spectrum animal imaging system (Perkin Elmer) was used for in vivo imaging.

Synthesis of probes

Synthesis of compound 1

A 250 mL round bottom flask was charged with 5-bromothiophene-2-

formaldehyde (1.91g, 10mmol), *p*-hydroxyphenyl boronic acid (1.38 g, 10 mmol), and tetra (triphenylphosphine) palladium (0.1 g, 0.1 mmol) in 90 mL anhydrous tetrahydrofuran, then 40 mL potassium carbonate aqueous solution (22% wt) was added quickly. Under the protection of nitrogen, the reaction mixture is stirred under reflux for 12 h and then quenched with water. The aqueous layer was extracted with dichloromethane and concentrated under reduced pressure to obtain a yellow oil. And the oil was finally purified by column chromatography (petroleum ether/ethyl acetate=1 /1, v /v) to obtain compound 4 (1.36 g, yield 67%). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.02 (s, 1H), 9.86 (s, 1H), 7.98 (d, *J* = 3.9 Hz, 1H), 7.56-7.66 (m, 3H), 6.87 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-*d6*) δ 184.1, 159.5, 154.2, 140.9, 140.0, 128.3, 123.9, 123.8, 116.6. ESI-MS: m/z 202.9 $[M-H]^-$.

Synthesis of compound 2

Compound 2 was synthesized according to previous work reported.[1]

Synthesis of compound DQMT-OH

Compound 1 (611 mg, 2.6 mmol), and compound 2 (461 mg, 2.2 mmol) were added into a 50mL round bottom flask, then added 15mL anhydrous acetonitrile and 15mL anhydrous ethanol as solvents, and added 250 μL piperidine as the catalyst. Under the protection of nitrogen, the reaction liquid is stirred for 12 h under reflux. After cooling down to room temperature, the resulting mixture evaporated the solvent under reduced pressure. Finally, compound DQMT-OH (664 mg, yield 72%) was obtained by column chromatography purification (dichloromethane/methanol=10 /1, v /v). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.82 (s, 1H), 8.88 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.9 Hz, 1H), 7.89-7.94 (m, 1H), 7.55-7.66 (m, 5H), 7.38 (d, 1H), 7.10 (d, 1H), 7.02 (s, 1H), 6.83 (d, *J*= 8.6 Hz, 2H), 4.55 (q, *J* = 6.9 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-*d6*) δ 158.2, 152.5, 148.9, 147.3, 138.3, 138.1, 134.1, 133.2, 133.1, 127.5, 125.6, 125.3, 124.6, 123.4, 121.0, 118.4, 118.2, 116.4, 106.9, 47.3, 29.4, 14.1. ESI-MS: m/z 420.1 $[M-H]^-$.

Preparation of standard solution

The system solutions used in the in vitro UV and fluorescence tests involved in the experiment are PBS solutions with pH = 7.4. Dissolve the probe DQMT-OH in

DMSO solution to prepare a standard solution with a concentration of 1mM; Dissolve sulfatase in PBS solution to prepare 100 U/mL standard solution, and dissolve other reaction objects, including metal ions, amino acids, and enzymes in PBS solution to prepare standard solution; Adjust the pH of the system solution with hydrochloric acid or sodium hydroxide to 3.0-10.0.

Fluorescence emission spectra of DQMT-OH dependent on sulfatase concentration

DQMT-OH (10 μM) Changes of the UV absorption spectrum and fluorescence emission spectrum in the mixed solution of water and DMSO with different water fractions (f_w). DQMT-OH (10 μM) in PBS solution (10 mM, pH 7.4, 1% DMSO) was treated with sulfatase of different concentrations (0-100 U/mL) for 30 minutes at 37℃ and then the fluorescence emission spectra of each mixed solution were measured with 440 nm excitation light, and the slit width was set as 10/10 nm.

Stability of DQMT-OH with the reaction system at different pH values

The reaction systems with different pH values were prepared, and only probe DQMT-OH (10 μM) was added. The fluorescence emission spectra of each solution were measured by excitation light at 440nm. The probe DQMT-OH (10 μM) and sulfatase (50 U/mL) were added into the reaction system at different pH values and incubated in a water bath thermostat at 37℃ for 30 min. The fluorescence emission spectra were measured on a fluorescence spectrophotometer.

Selectivity of DQMT-OH with different analytes in PBS

The fluorescence emission spectra of DQMT-OH (10 μM, 1% DMSO, pH 7.4) with different analytes in PBS were determined by fluorescence spectrophotometer. The concentrations of Na^+, Mg^{2+}, K^+, I^-, CO_3^{2-}, CH_3COO^-, HPO_4^{2-}, HCO^{3-}, H_2PO^{4-}, SO_4^{2-} were 10 μM. The concentrations of glutathione (GSH), cysteine (Cys), Glycine (Gly), dithiothreitol (DTT), vitamin C, glutamic acid (Glu), and arginine (Arg) were 100 μM. The concentrations of bovine serum albumin (BSA), glucose oxidase (GOD), catalase (CAT), trypsin, *β*-glucosidase (β-GLC), and β-galactosidase (β-GAL) were 100 μg/mL. The concentration of sulfatase (SULF) was 50 U/mL. All different analytes in PBS were added into DQMT-OH and then were incubated in a water bath for 30 min at 37℃.

Molecule docking studies

Molecular docking simulations were conducted with the Molecular Operating Environment (MOE) software version 2014.0901 (Chemical Computing Group, Montreal, Canada). The crystal structures of sulfatase (PDB ID: 4CXU) were obtained from the Protein Database (www. rcsb.org). Hydrogen atoms were added to the protein and energy minimization was performed using the MMFF94 force field. Docking of the ligand DQMT-OH into the active site of the sulfatase was performed by the triangle matcher docking protocol of the MOE program. As the refinement method, the force field was chosen. The resulting poses are then scored using the GBVI/WSA dG docking scoring function. The figures were generated using Discovery Studio 2019 Client.

Cell culture

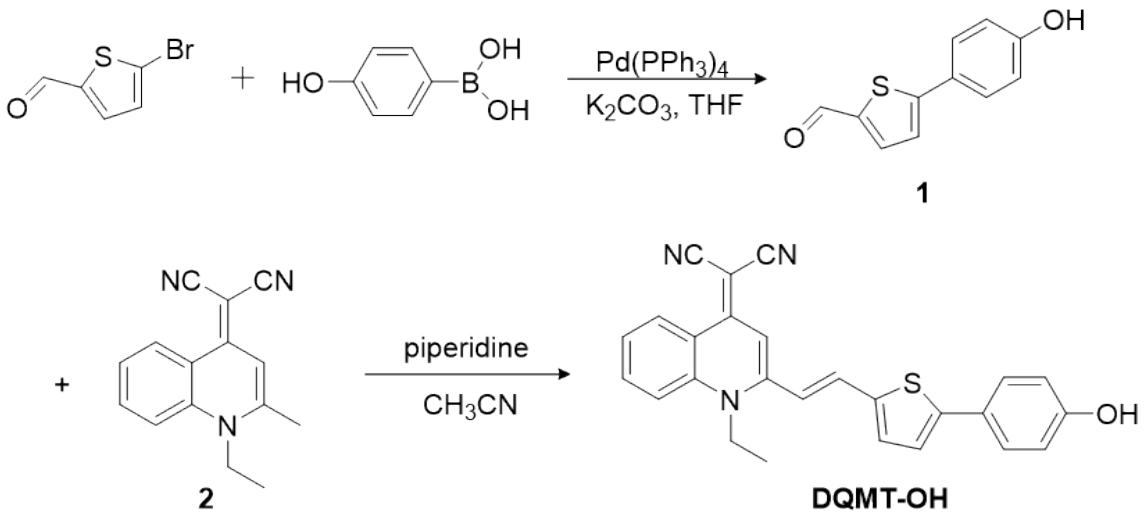
The L02 cells, 4T1 cells, and MCF-7 cells were purchased from KGI Biotechnology Co., LTD. (Nanjing, China). All cells were cultured in DMEM medium containing 10% fetal bovine serum and antibiotics (FBS) and antibiotics (100U/mL penicillin and streptomycin), maintained at 37℃ in a humidified atmosphere of 95% air and 5% CO_2.

Cell viability assay

The toxicity of MCF-7 cells and 4T1 cells was studied by using the standard MTT assay. Cells at the logarithmic growth stage were transferred to a different 96-well plate (1×10^5 cells per well) and cultured at 37℃ and 5% CO_2 cell incubator for 24 h. The probes were separately added to plates at different concentrations (0, 5, 10, 15, 20, and 30 μM) and cultured in an incubator (37°C, 5% CO_2) for 24 h. Then MTT solution (10 μL, 5.0 mg/mL) was added to each well. The 96-well plate was taken out of the incubator after 4 h. The medium containing MTT was removed and DMSO (150 μL) was added to each well. After shaking for 15 min on a shaker, absorbance was measured at 490 nm with the enzyme-labeling measuring instrument. Each concentration was measured in quadruplicate and used in four independent experiments. The relative cell viability was calculated by the equation: cell viability (%) = $(OD_{treated} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100\%$.

In vivo fluorescence imaging

The animals involved in the experiment were purchased from Qinglongshan Animal Breeding Center in Nanjing, Jiangsu Province. All animals have passed the quality test of China Pharmaceutical University before use and are operated in strict accordance with the management regulations of the National Animal Protection Center.



Scheme S1. Synthetic route of probe DQMT-OH

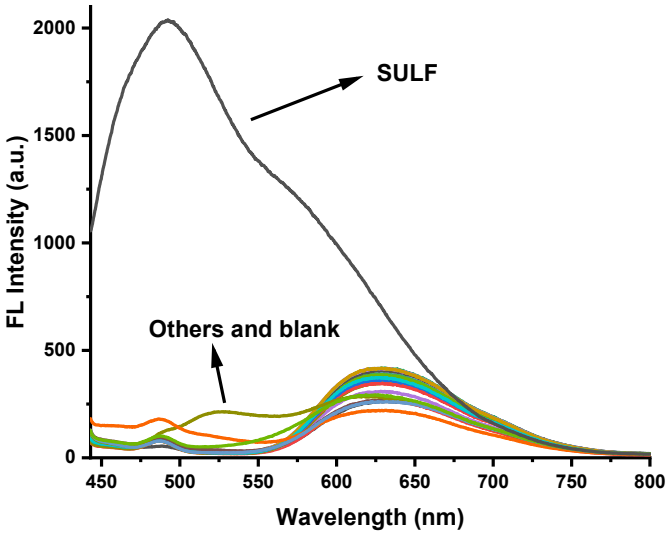


Figure S1. Fluorescent spectra of DQMT-OH (10 μM) with SULF and other analytes encompassed: (0) blank; (1) Na^+; (2) Mg^{2+}; (3) K^+; (4) I^- ; (5) CO_3^{2-}; (6) CH_3COO^- ; (7) HPO_4^{2-}; (8) HCO_3^-; (9) $H_2PO_4^-$; (10) SO_4^{2-}; (11) GSH; (12) Cys; (13) Gly; (14) DTT; (15) Vitamin C; (16) Glu; (17) Arg; (18) BSA; (19) GOD; (20) CAT; (21) Trypsin; (22) *β*-GLC; (23) *β*-GAL; (24) SULF.

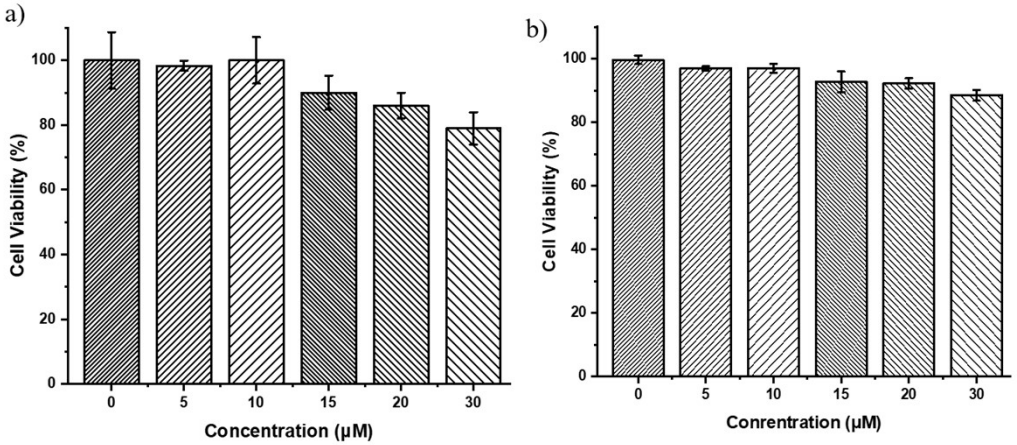


Figure S2. Effect of different concentrations (0-30 μM) of DQMT-OH on the viability of a) MCF-7 cells and b) 4T1 cells at 24 h. Data are expressed as the mean ± SD of three parallel experiments.

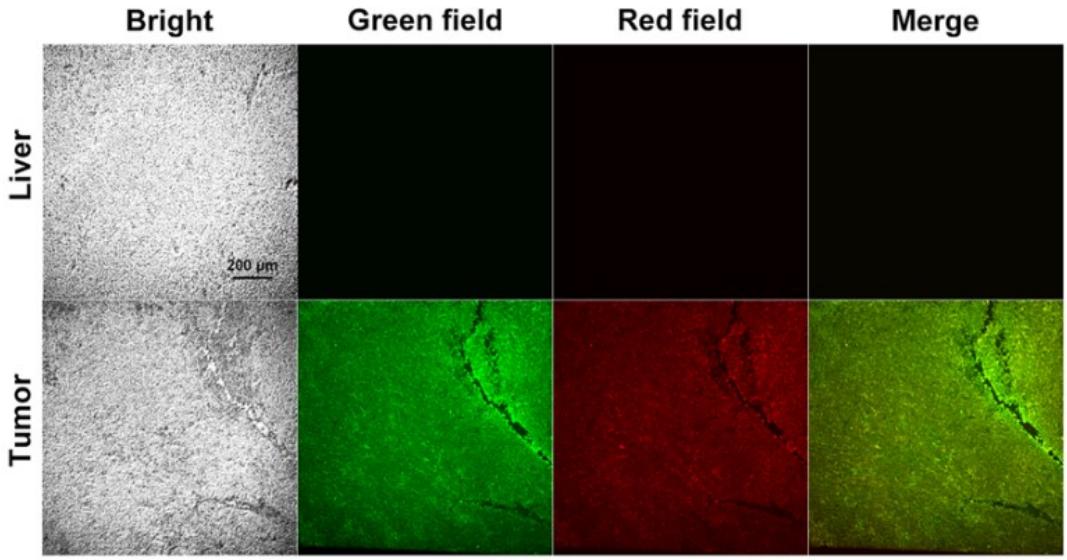


Figure S3. Images of liver tissue and tumor tissue.

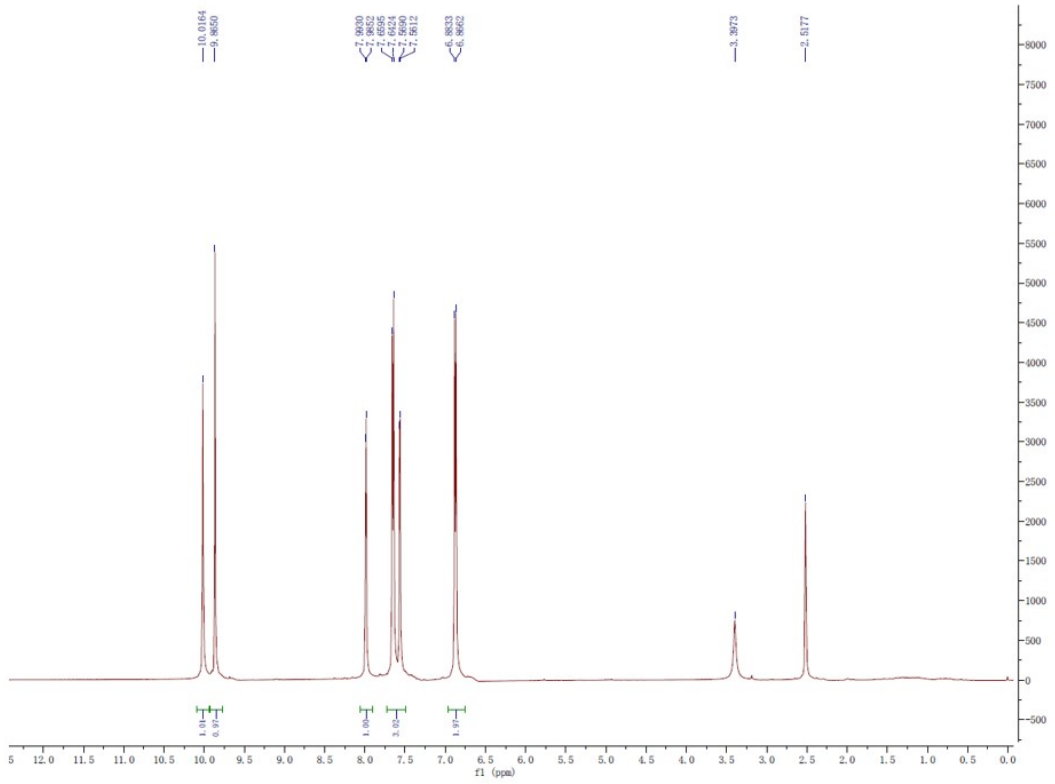


Figure S4. ^{1}H NMR of compound 1.

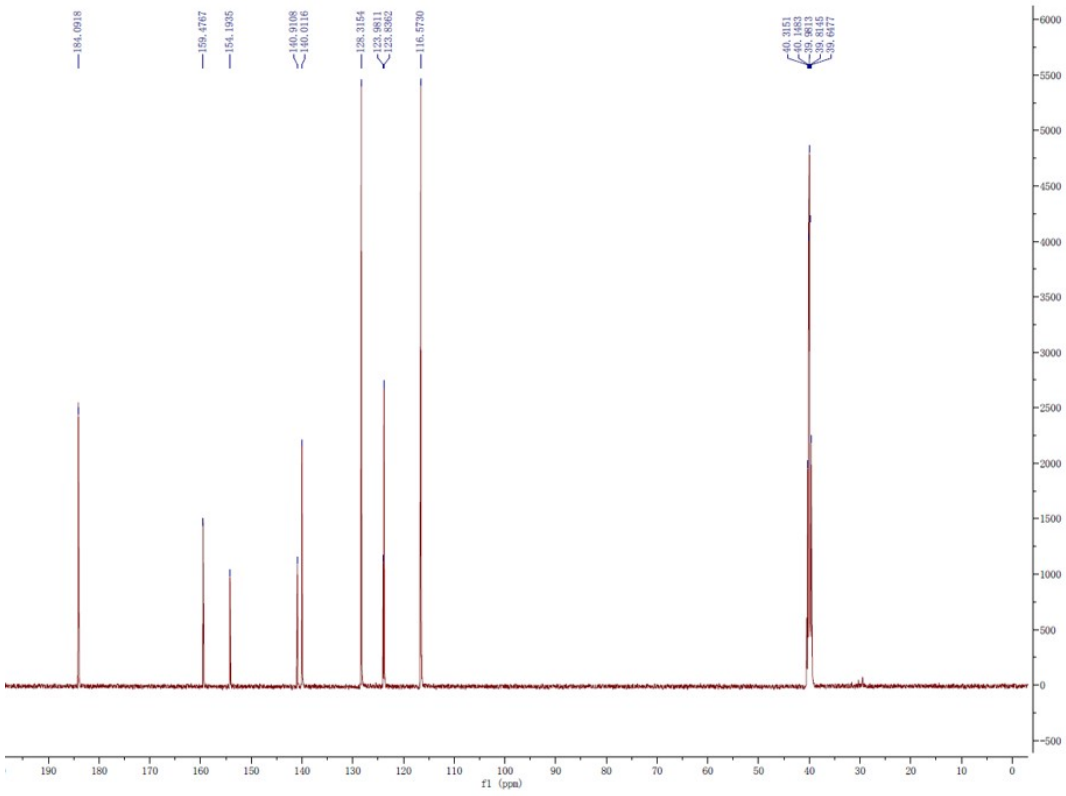


Figure S5. ^{13}C NMR of compound 1.

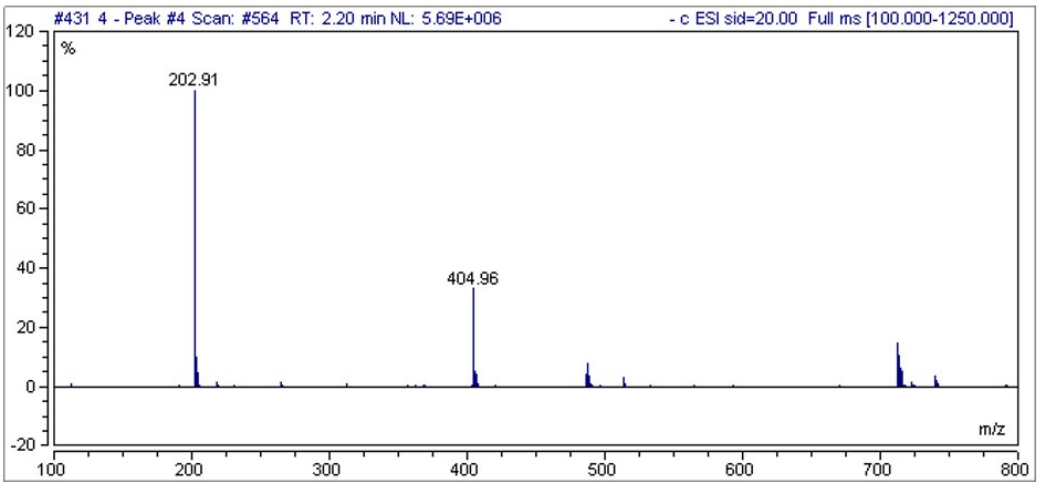


Figure S6. HRMS of compound 1.

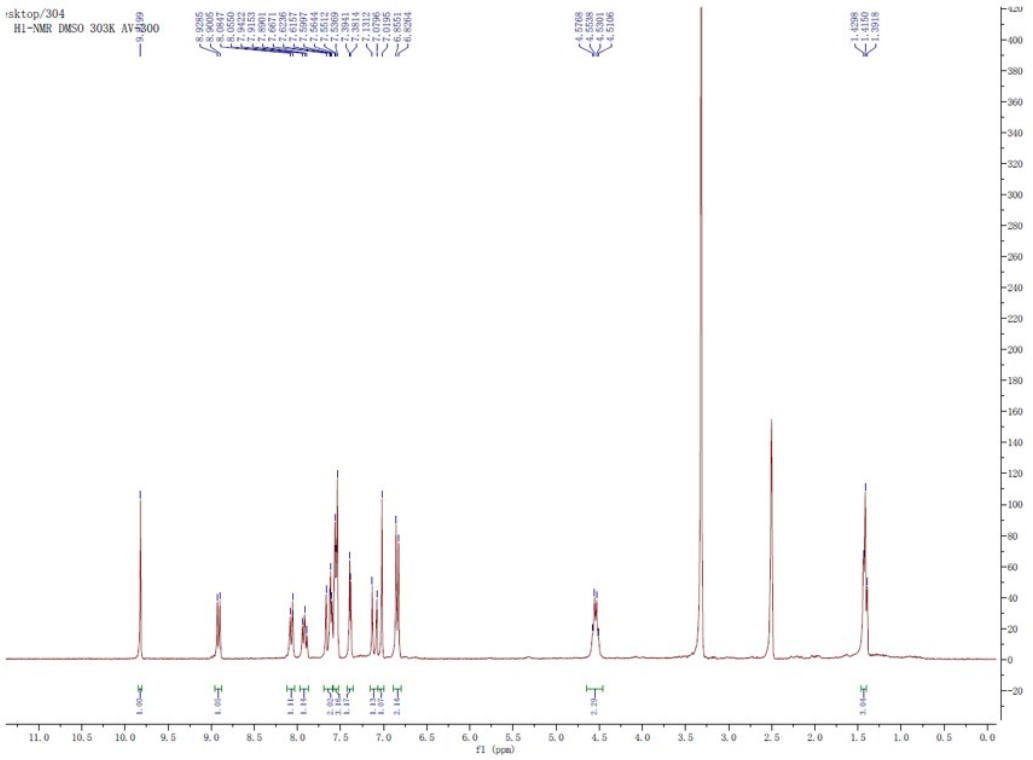


Figure S7. ^{1}H NMR of DQMT-OH.

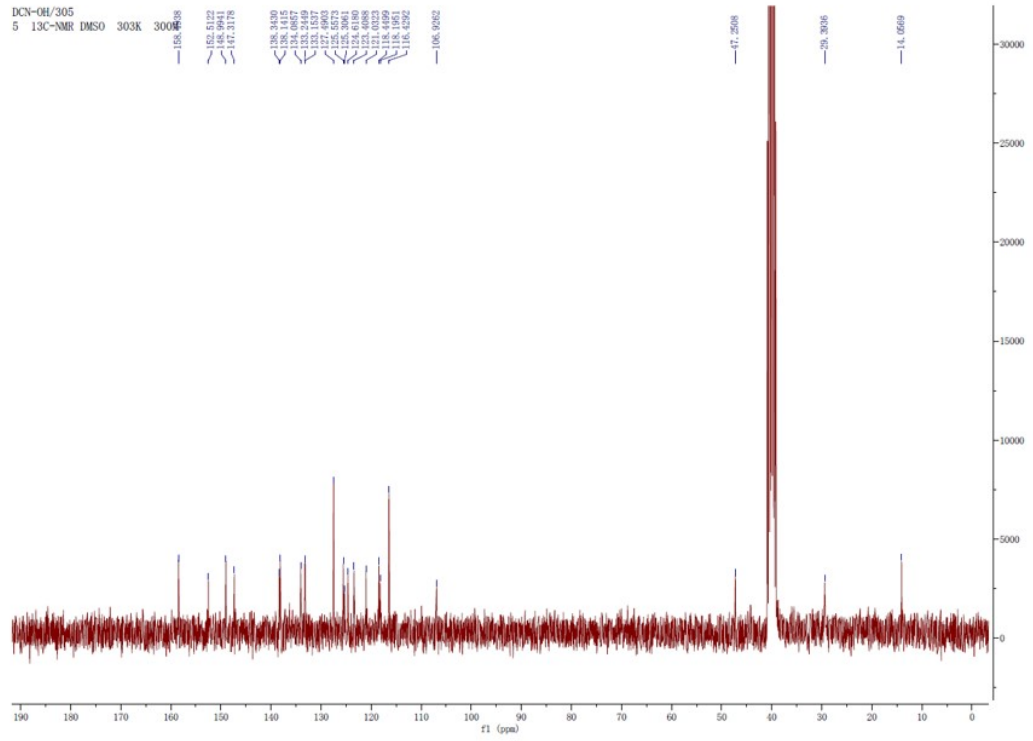


Figure S8. ^{13}C NMR of DQMT-OH.

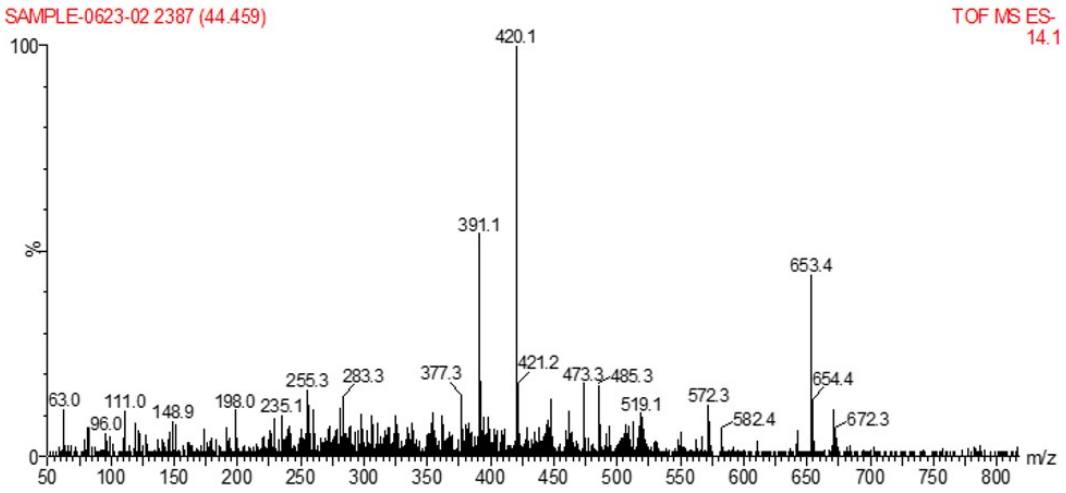


Figure S9. MS of DQMT-OH.

Reference:

1. Xu, Y.; Cui, M.; Zhang, W.; Liu, T.; Ren, X.; Gu, Y.; Ran, C.; Yang, J.; Wang, P., A sulfatase-activatable AIEgen nanoprobe for inhalation imaging-guided surgical excision of lung cancer. *Chemical Engineering Journal* **2022,** *428*, 132514-.