Electronic Supplementary Information (ESI) for

A novel fluorescent probe for selective detection of thiols in acidic solutions and labeling of acidic organelles in live cells

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I. Experimental Details

General methods. All the chemicals for synthesis were purchased from commercial suppliers and were used as received without further purification. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ or [D_6]DMSO with a Bruker AV spectrometer operating at 300 MHz or 400 MHz and 100 MHz, respectively and chemical shifts were reported in ppm using tetramethylsilane (TMS) as the internal standard. FT-IR spectra were measured with a Bruker Vector22 Infrared Spectrometer. Mass spectra were obtained with a Thermo LTQ Orbitrap mass spectrometer. UV-vis absorption and fluorescence emission spectra were recorded with a Shimadzu UV-2450 UV/vis spectrometer and a Perkin-Elmer Instruments LS55 Luminescence Spectrometer, respectively. High-performance liquid chromatography (HPLC, Agilent 1200 series) was employed for the analysis and the separation of adducts of probes with Cys.

Preparation of sample solutions. Sample solutions for measurements were DMSO/buffer (v/v 2:98) solvent mixtures (0.1 M citric acid–0.1 M disodium hydrogen phosphate buffer for pH values ranging from 2 to 8, and 0.1 M Na_2CO_3–0.1 M $NaHCO_3$ buffer for pH 9–11). Water for sample solution preparation was purified with a Millipore water system. All pH values were measured on a MQK PHS-3C pH meter.

Cell culture and images. MDA-MB-231 cells were seeded on the coverslips in 24-well plates at a density of 50000 cells/well and incubated in a humidified 5% CO_2 atmosphere for 24h with the complete Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum at 37ºC. DMSO solution of QMA or the ester derivative QME was added to a well to give concentration of 20μM and volume ratio of DMSO/culture medium is 1/1000. After incubated for 3h, cells were further stained with LysoTracker® Red (Molecular Probe, Eugene, OR) following the manufacturer's instructions. The 1 mM stock solution of LysoTracker® Red was prepared, and the final working concentration of 75 nM in the DMEM medium. The medium was removed from the dish, and then the LysoTracker® Red containing medium was added. Incubate the cells for 45 min under growth conditions, and then the cells were washed twice with PBS buffer after culture medium was removed. The cells were then fixed with fresh 4% paraformaldehyde for 10 min at room temperature and washed with PBS buffer twice. The coverslips were mounted on the glass microscope slides with a

drop of anti-fade mounting media (Sigma-Aldrich, USA) to reduce fluorescence bleaching. The cellular localization was visualized under a laser scanning confocal microscope (LSM 710 Meta, Carl Zeiss Inc., Thornwood, NY). The red fluorescence of cells was collected with 600–750 nm channel under excitation at 530 nm, and green fluorescence of cells was collected with 500–575 nm channel under two-photon excitation at 750 nm.

Kinetic analysis. Time-dependent response of QMA to different excess thiols exhibits pseudo-first-order reaction conditions. The rate constant k_{obs} is obtained according to the equation:

$$\ln[(A - A_{min})/(A_0 - A_{min})] = -k_{obs}t$$

where A_0, A and A_{min} are absorption values at certain wavelength, where thiols have no absorption, of the solution before and after addition of thiols, and the corresponding adduct or after complete reaction of probe molecules, respectively. Furthermore, a second-order rate constant k was obtained according to the equation, $k_{obs} = k$[Cys], where [Cys] is the concentration of thiols.

II. Related analysis results

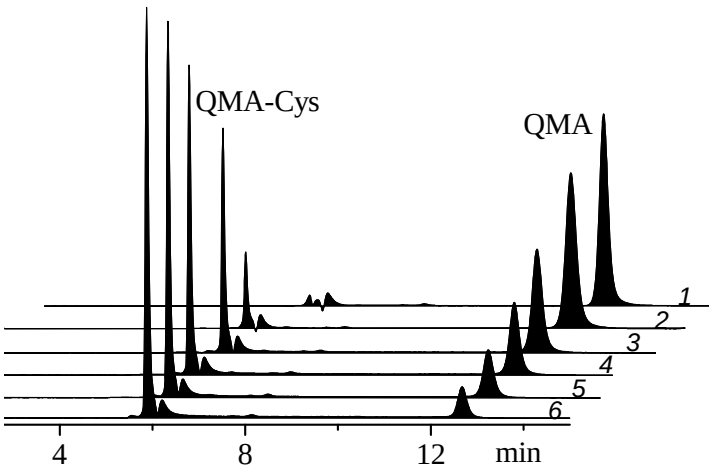


Figure S1. Typical HPLC chromatogram obtained from the reaction of QMA with different amount of Cys: 1, 0 equiv.; 2, 0.5 equiv.; 3, 1 equiv.; 4, 1.5 equiv.; 5, 2.5 equiv.; 6, 3.5 equiv, in DMSO/H_2O (v/v 2:98, pH 3.8) solution. Assay conditions: C_{18}-reversed phase column, 50% methanol and 50% 5 mM H_2SO_4 as eluent, detection wavelength, 360 nm.

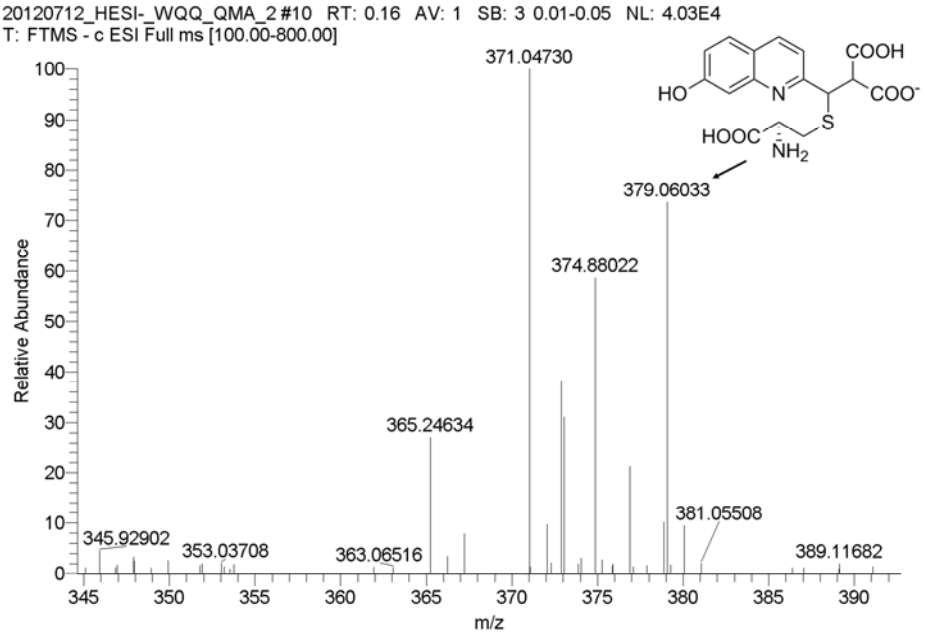


Figure S2. Mass spectrum of the reaction mixture of QMA with Cys·HCl in DMSO.

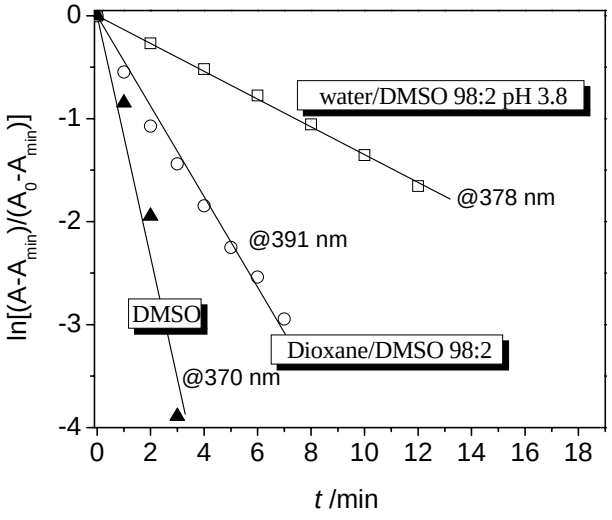


Fig. S3 Pseudo-first-order profile (k_{obs}) of QMA (20 μM) with 1.5 equiv. of MAA in three solvents based on the absorbance at the showing wavelength.

III. Synthesis and characterization of related compounds

2-methylquinolin-7-yl acetate (1). Acetic anhydride (0.83 g, 8.2 mmol) was added dropwise into a 20 mL CH_2Cl_2 solution of 2-methylquinolin-7-ol (0.65 g, 4.1 mmol) within 10 min, and then stirred for 4 h at room temperature. The reaction mixture was washed with saturated $NaHCO_3$ solution twice, and removed solvent *in vacuo* afforded **1** (0.51 g, 62%) as light-brown oil. R_f = 0.62 (ethyl acetate / petroleum ether 1:3); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 2.36 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 7.27–7.29 (m, 2H, quinoline-H), 7.76–7.79 (m, 2H, quinoline-H), 8.06 ppm (d, ^3J(H,H)=8.4 Hz, 1H, quinoline-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ = 169.3 (C=O), 159.8, 151.5, 148.0, 136.3, 128.6, 124.5, 121.9, 121.3, 119.4, 25.0 (CH_3), 21.2 ppm (CH_3); IR (Nujol): νbar = 1763 (OC=O), 1625, 1509, 1430, 1371, 1202 cm^{-1}; FTMS (APCI) calcd for $C_{12}H_{12}NO_2$: 202.0868 ([M+H]$^+$), found 202.0860.

2-formylquinolin-7-yl acetate (2). 1 (0.51 g, 2.5 mmol) was dissolved in 2 mL dioxane, and then added to 3mL dioxane suspension with 0.43 g SeO_2. The mixture reacted at 90°C for 6 h under N_2 atmosphere, and 30 mL ethyl acetate was added for dilution, followed by washing with water once. Solvents were removed in vacuo, and purification by column chromatography (silica gel-H, ethyl acetate / petroleum ether 1:3) gave **2** (0.35 g, 64%) as a light-yellow powder. R_f = 0.69 (ethyl acetate / petroleum ether 1:3); m.p. 143–144°C; ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 2.40 (s, 3H, CH_3), 7.47 (d, ^3J(H,H)=7.5Hz, 1H, quinoline-H), 7.80–8.03 (m, 3H, quinoline-H), 8.32 (d, ^3J(H,H)=8.1Hz, 1H, quinoline-H), 10.22 ppm (s, 1H, HC=O); ^{13}C NMR (100 MHz, [D_6]DMSO, 25˚C, TMS): δ = 193.6 (C=O), 169.0 (COO), 152.8, 151.9, 147.7, 137.9, 129.5, 127.6, 125.1, 120.6, 116.9, 20.9 ppm (CH_3); IR (Nujol): νbar = 2853, 1760 (OC=O), 1706 (C=O), 1434, 1368, 1211 cm^{-1}; FTMS (APCI) calcd for $C_{12}H_{10}NO_3$: 216.0661 ([M+H]$^+$), found 216.0652.

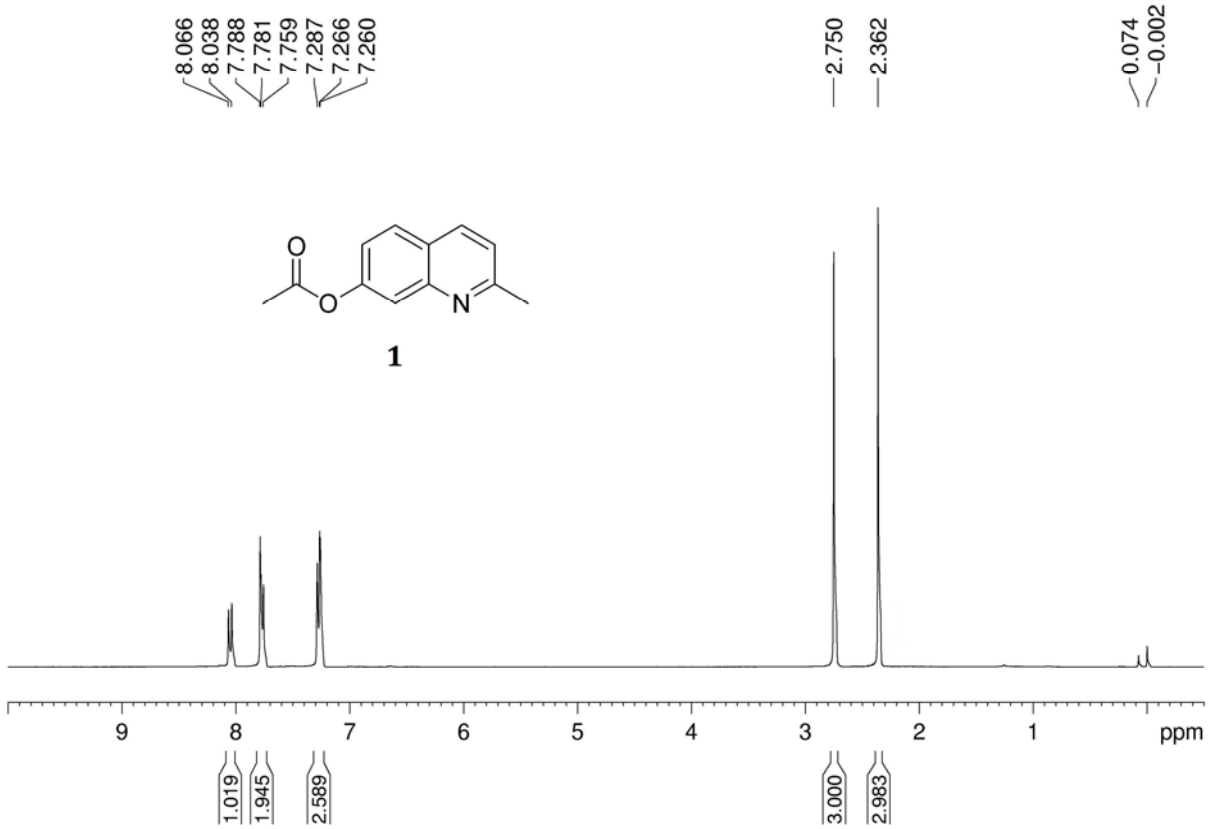
7-hydroxyquinoline-2-carbaldehyde (3). Sodium methanolate (0.17 g, 3.2 mmoL) was added to a 10 mL solution of **2** (0.35 g, 1.6 mmoL) in methanol and then stirred at room temperature for 0.5 h. 20 mL water was added. The reaction mixture was acidified with acetic acid to pH about 6.0, followed by extraction four times with ethyl acetate (4×5 mL). Solvent in the extractive was removed under reduced pressure to yield **3** (0.25 g, 89%) as a yellow powder. R_f = 0.50 (ethyl acetate); m.p. 150–152°C; ^{1}H NMR (400 MHz, [D_6]DMSO, 25°C, TMS): δ = 7.34-7.37 (m, 1H, quinoline-H), 7.42

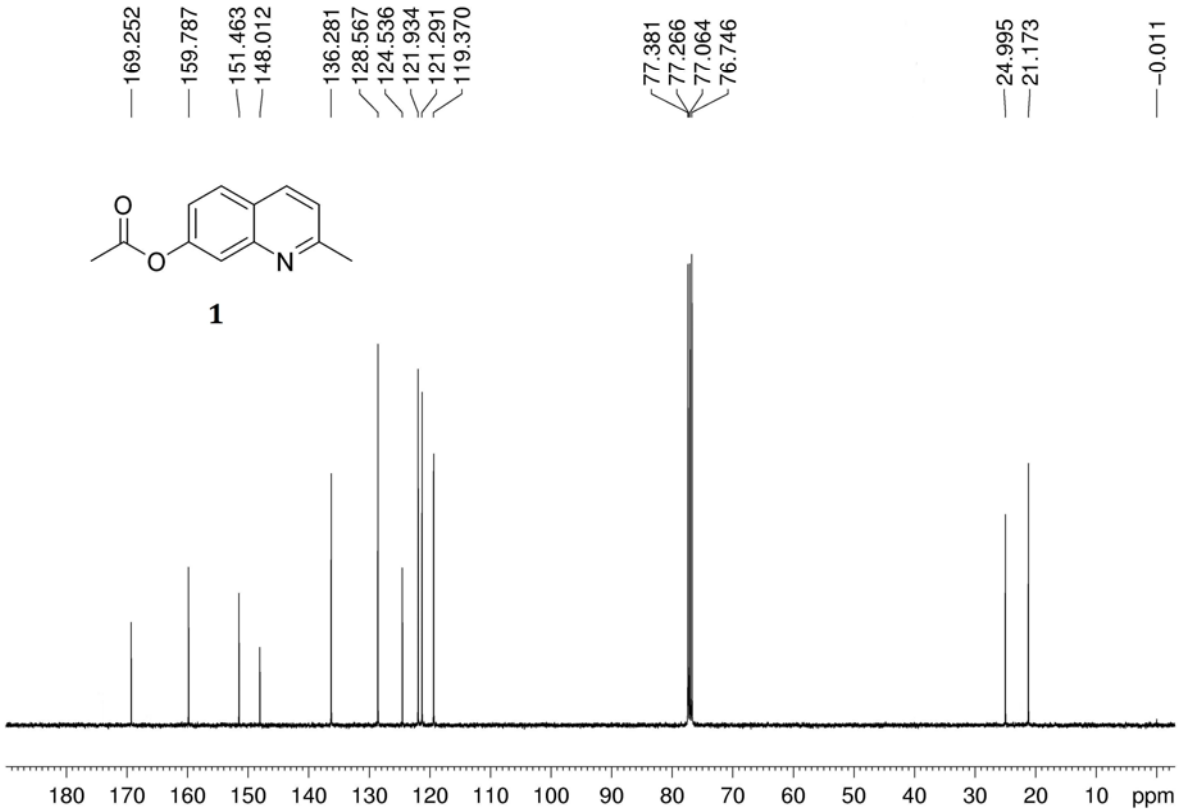
(s, 1H, quinoline-H), 7.77 (d, ^{3}J(H,H)=8.4Hz, 1H, quinoline-H), 7.97 (d, ^{3}J(H,H)=8.8Hz, 1H, quinoline-H), 8.44 (d, ^{3}J(H,H)=8.4Hz, 1H, quinoline-H), 10.08 (s, 1H, OH), 10.50 ppm (s, 1H, HC=O); ^{13}C NMR (100 MHz, [D_6]DMSO, 25°C, TMS): δ = 194.0 (C=O), 159.5, 152.4, 149.2, 137.4, 129.5, 124.1, 122.1, 114.1, 110.4 ppm; IR (Nujol): *v*bar = 3391 (O-H), 1708 (C=O), 1627, 1446, 1296, 1254 cm^{-1}; FTMS (APCI) calcd for $C_{10}H_8NO_2$: 174.0555 ([*M*+H]$^{+}$), found 174.0547.

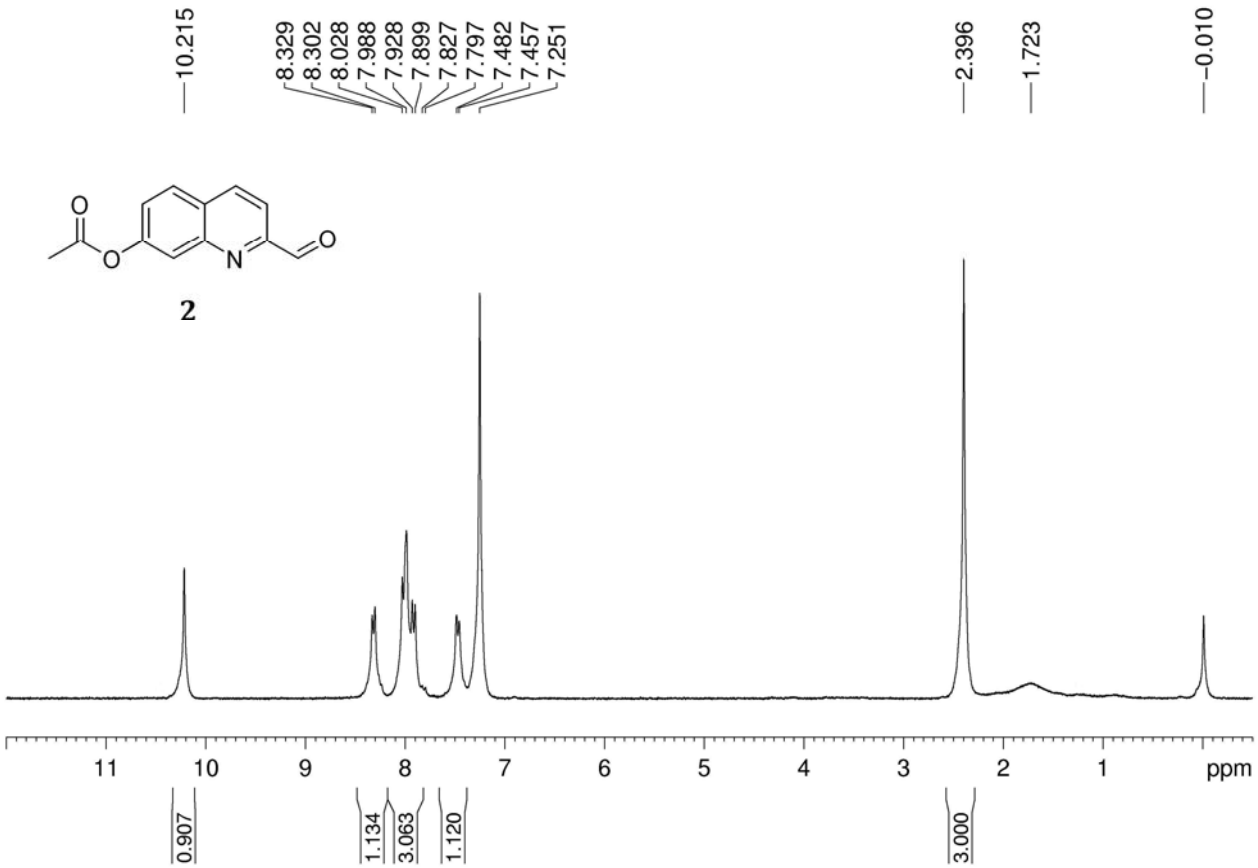
2-((7-hydroxyquinolin-2-yl)methylene)malonic acid (QMA). 3 (0.15 g, 0.87 mmol) was added to the solution of malonic acid (90 mg , 0.87 mmol) in ethanol (6 mL) and stirred at 50˚C for 30 minutes in the presence of piperidine of catalytic amount (10 mol%) under N_2 atmosphere. The precipitate was filtered, washed three times with ethanol (3 × 5 mL) and dried to give QMA (0.14 g, 64%) as a orange powder. m.p. >200°C; ^{1}H NMR (300 MHz, [D_6]DMSO, 25°C, TMS): δ = 7.18–7.24 (m, 2H, quinoline-H), 7.56 (d, ^{3}J(H,H)=8.4Hz, 1H, quinoline-H), 7.65 (s, 1H, double bond-H), 7.85 (d, ^{3}J(H,H)=9.0Hz, 1H, quinoline-H), 8.32 (d, ^{3}J(H,H)=8.4Hz, 1H, quinoline-H), 10.38 (s, 1H, OH), 13.45 ppm (br, 2H, COOH); ^{13}C NMR (100 MHz, [D_6]DMSO, 25°C, TMS): δ = 167.3 (COO), 165.3 (COO), 159.3, 151.1, 148.5, 137.3, 137.0, 131.7, 129.2, 122.2, 120.8, 120.0, 109.3 ppm; IR (Nujol): *v*bar = 3436 (O-H), 1698, 1619, 1593, 1416 cm^{-1}; FTMS (APCI) calcd for $C_{13}H_{10}NO_5$: 260.0559 ([*M*+H]$^{+}$), found 260.0552.

Diethyl 2-((7-hydroxyquinolin-2-yl)methylene)malonate (QME). 3 (0.15 g, 0.87 mmol) was added to the solution of malonic acid diethyl ester (0.14 g, 0.87 mmol) in ethanol (8 mL) and stirred at 50°C overnight in the presence of piperidine of catalytic amount (10 moL%) under N_2 atmosphere. Solvent in the reaction mixture was removed and the crude product was subjected to column chromatography (silica gel-H, ethyl acetate / petroleum ether 1 : 3) to afford QME (40 mg, 15%) as a light-yellow powder. R_f = 0.48 (ethyl acetate/ petroleum ether 1:1); m.p. 123–125°C; ^{1}H NMR (300 MHz, $DCCl_3$, 25°C, TMS): δ = 1.35–1.41 (m, 6H, CH_3), 4.32-4.39 (q, ^{3}J(H,H)=7.2 Hz, 2H, CH_2), 4.53–4.60 (q, ^{3}J(H,H)=7.2 Hz, 2H, CH_2), 7.05 (d, ^{3}J(H,H)=9.0 Hz, 1H, quinoline-H), 7.25–7.33 (m, 2H, quinoline-H), 7.55 (d, ^{3}J(H,H)=9.0Hz, 1H, quinoline-H), 7.80 (s, 1H, double bond-H), 8.01 ppm (d, ^{3}J(H,H)=8.4Hz, 1H, quinoline-H); ^{13}C NMR (100 MHz, $DCCl_3$, 25˚C, TMS): δ = 168.3 (COO), 163.8 (COO), 158.4, 150.5, 149.0, 140.0, 136.5, 129.2, 128.5, 123.0, 120.6, 120.4, 110.8, 61.9 (CH_2), 14.1 ppm (CH_3); IR (Nujol): *v*bar = 3435 (O-H), 1719, 1618, 1509, 1454, 1390 cm^{-1}; FTMS (APCI) calcd for $C_{17}H_{18}NO_5$: 316.1185 ([*M*+H]$^{+}$), found 316.1179.

IV. ^{1}H NMR and ^{13}C NMR spectra of new compounds



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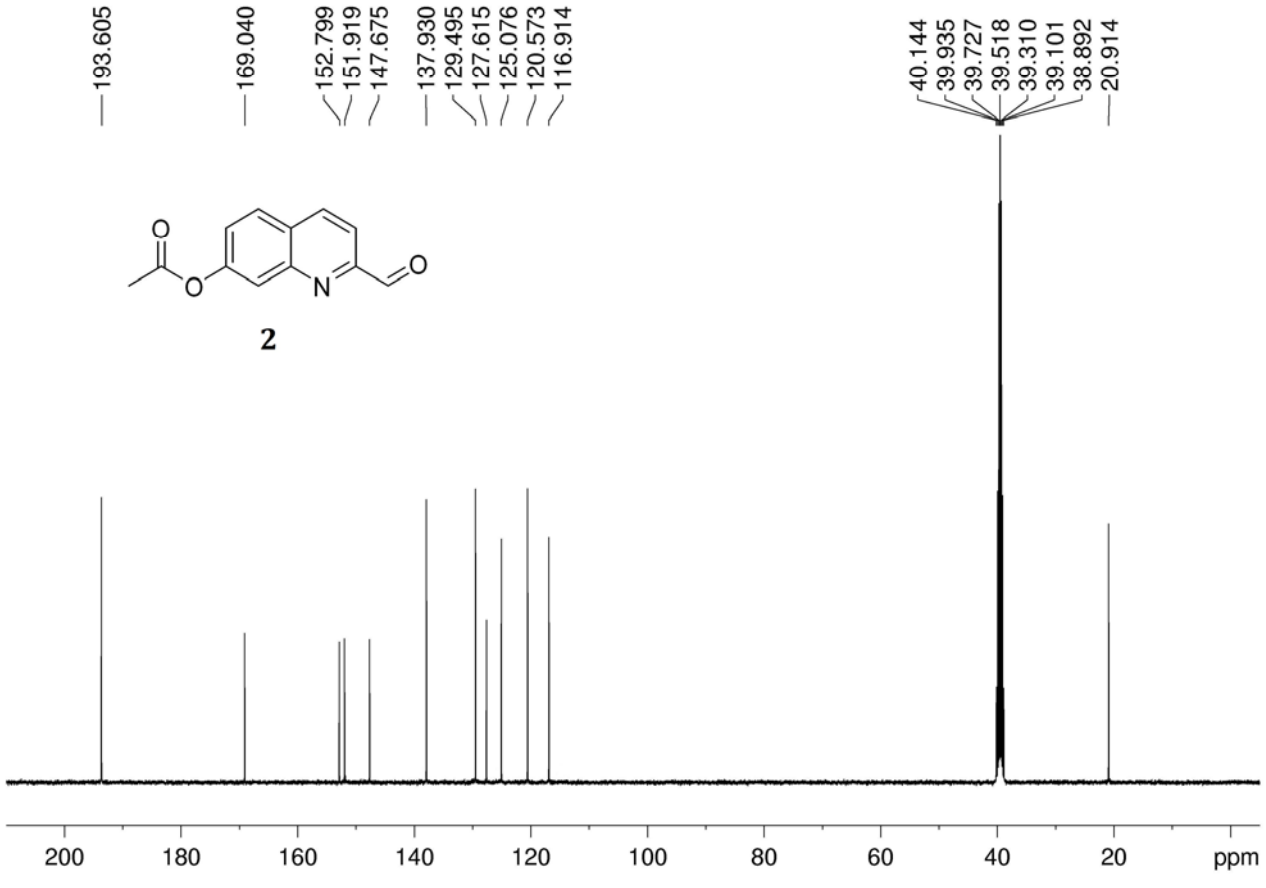
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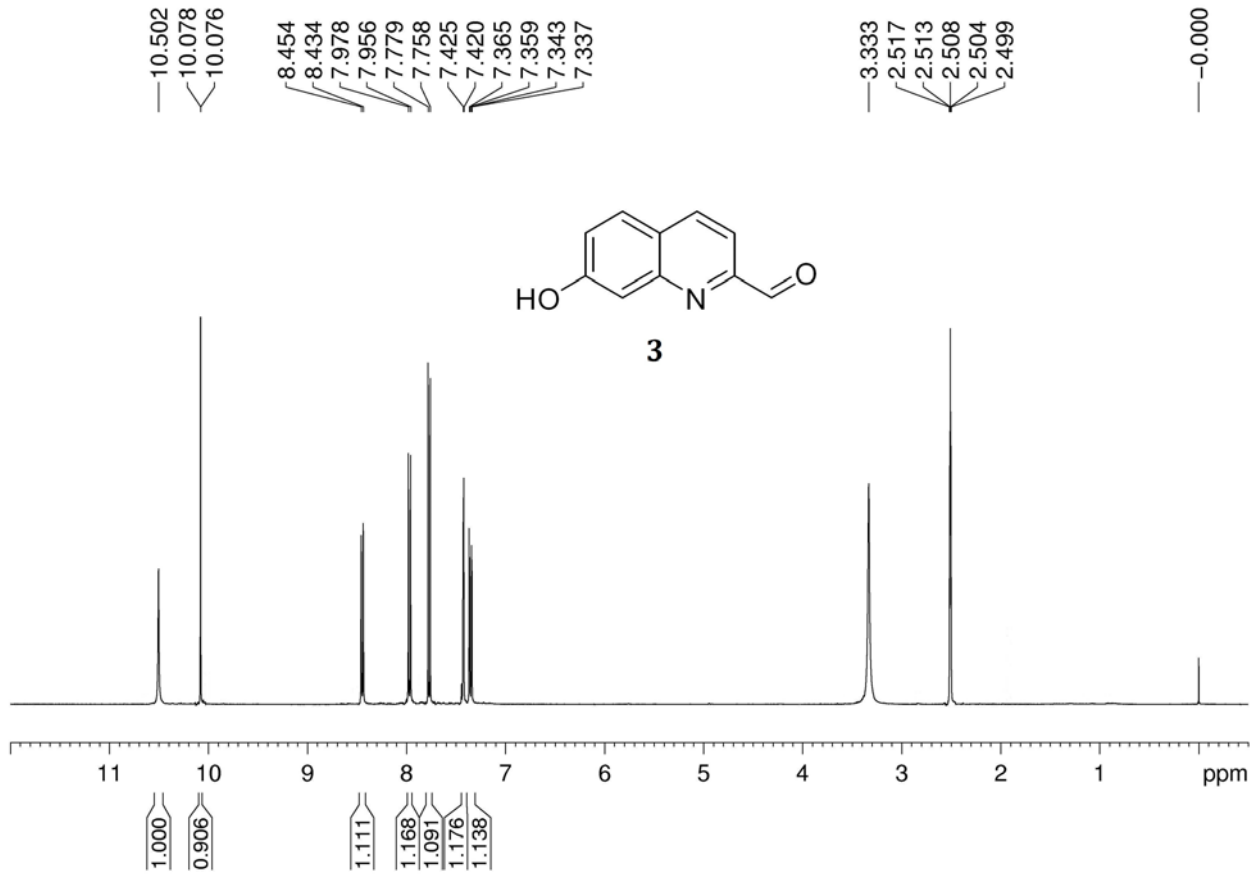

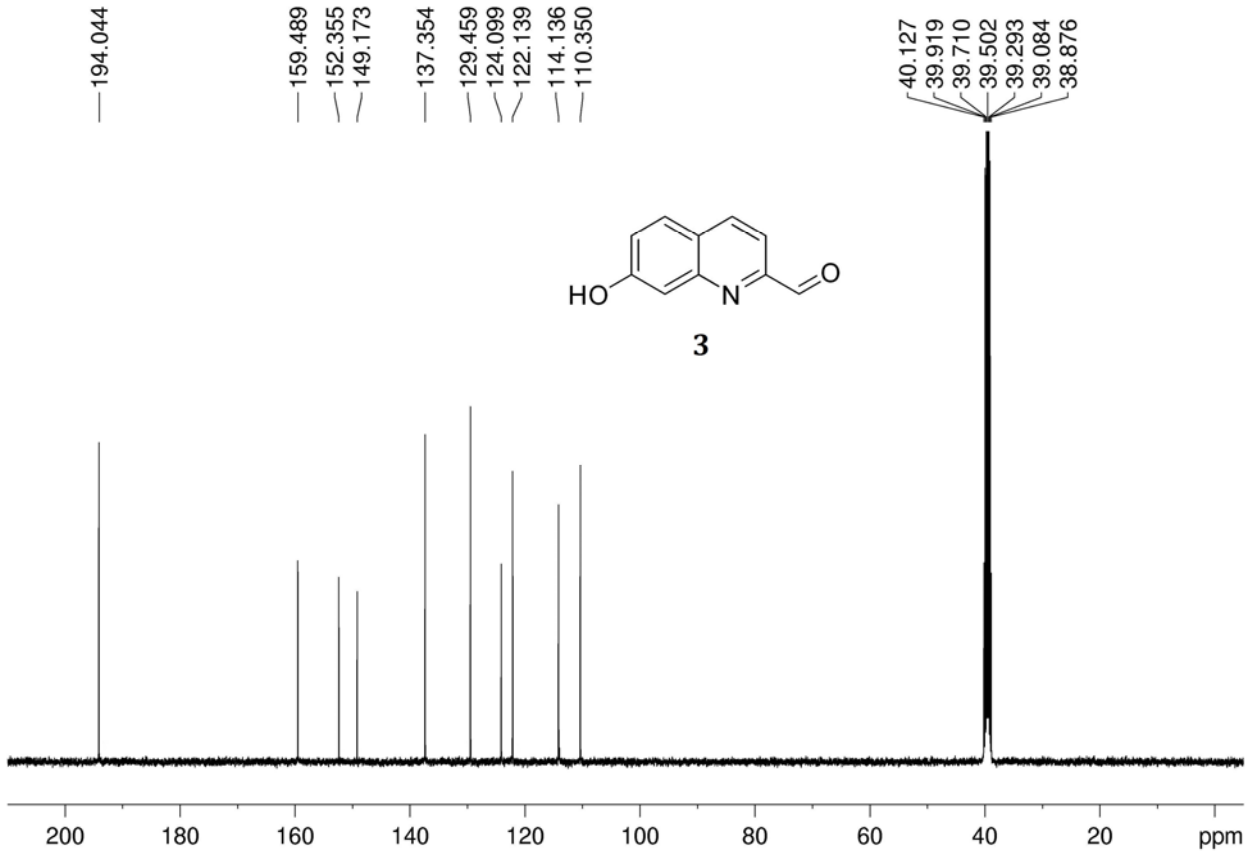

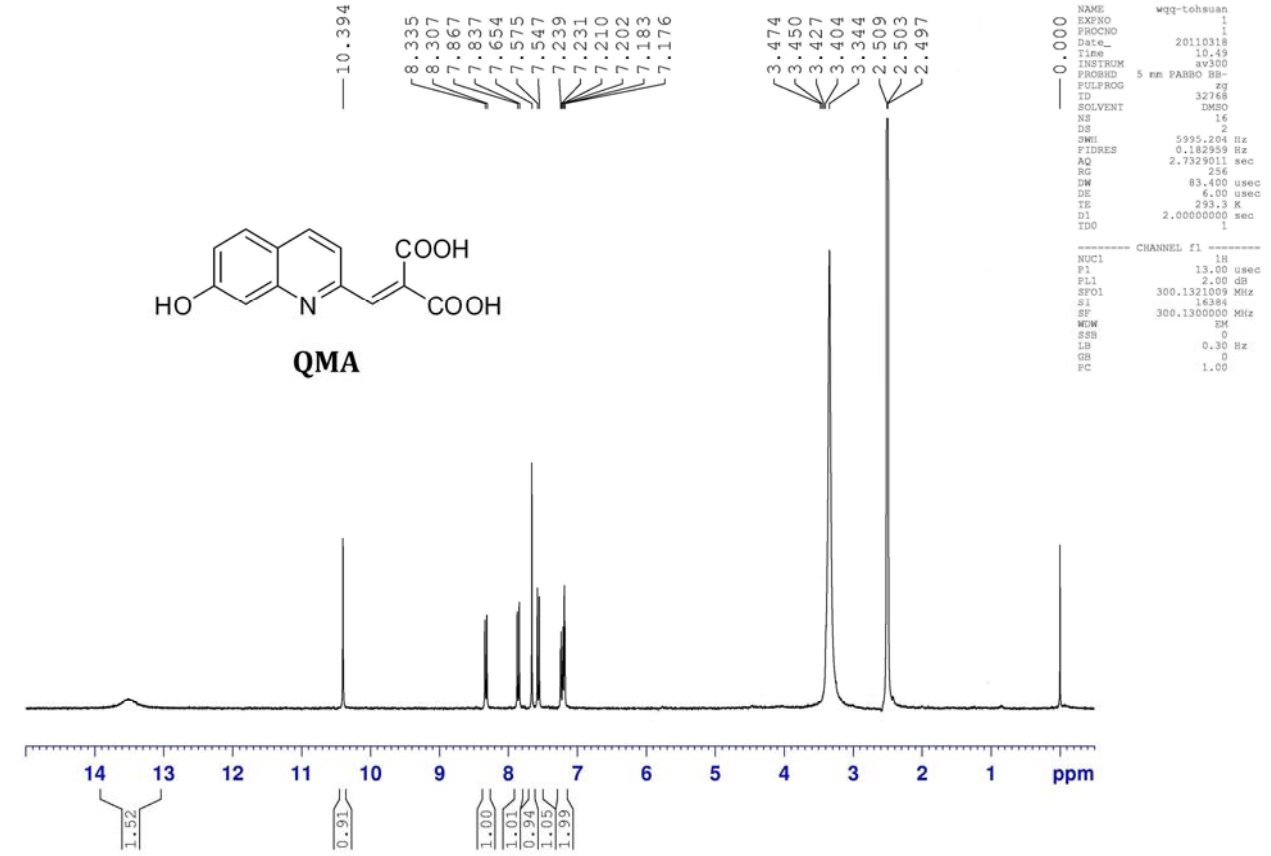

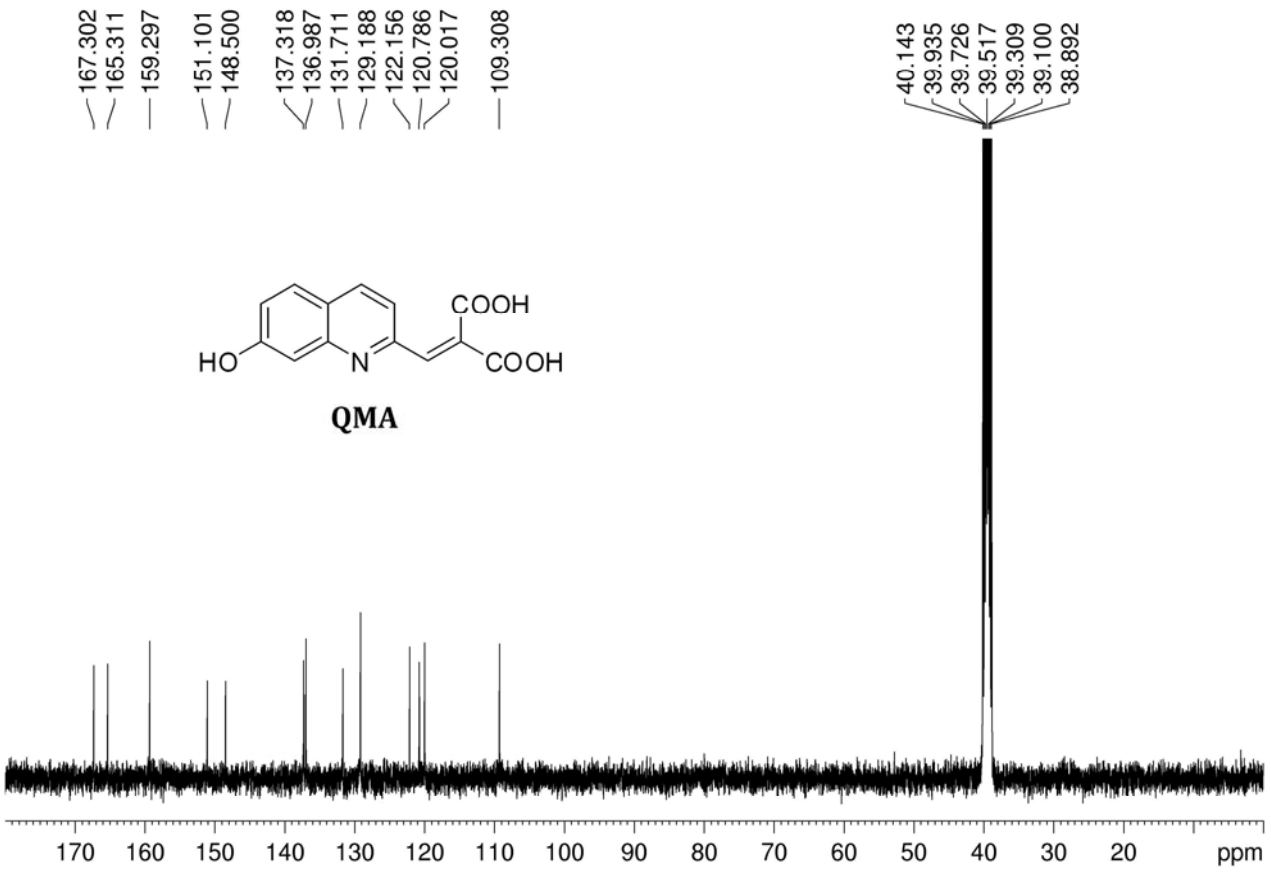

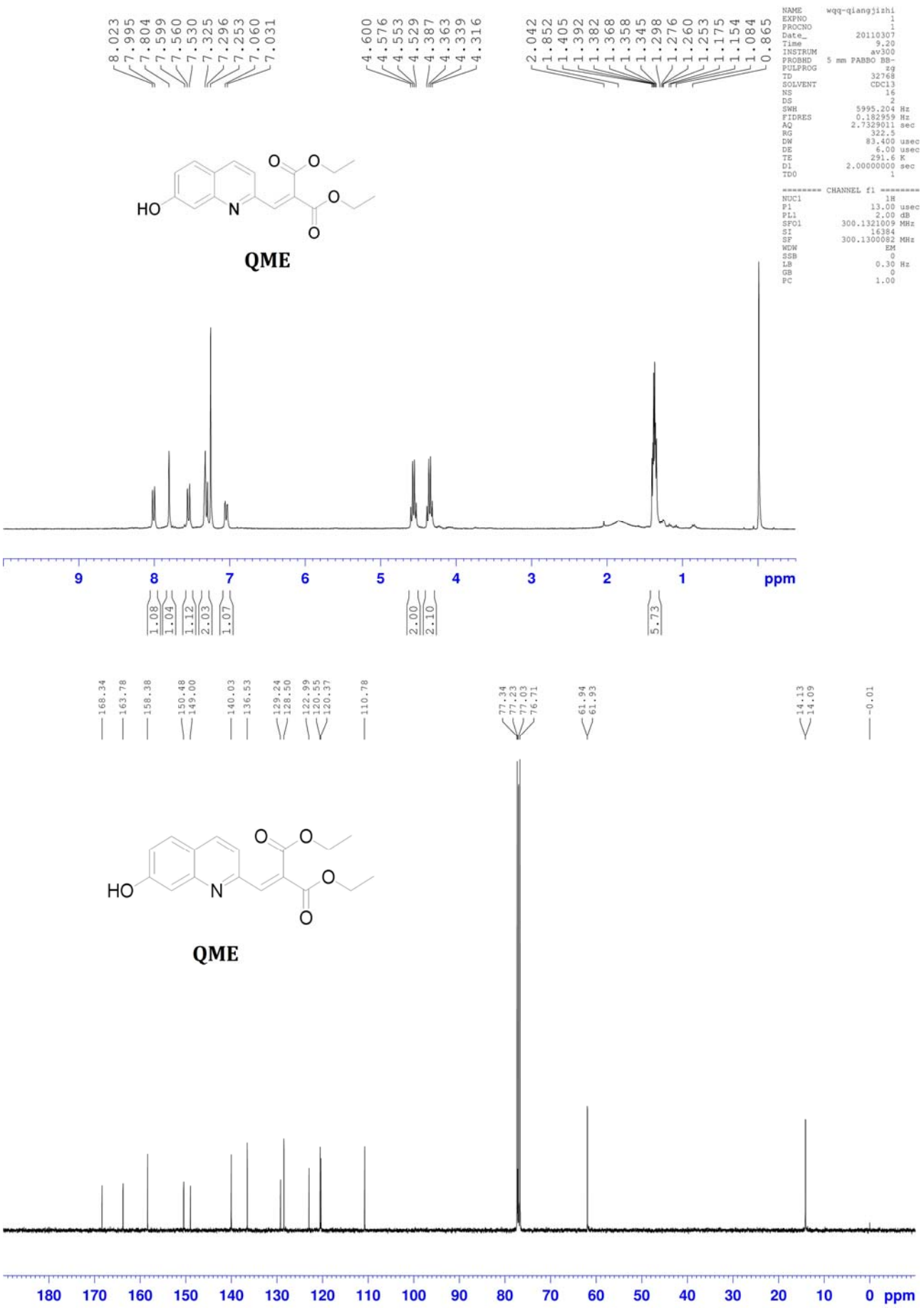
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