Electronic Supplementary Material (ESI) for Chemical Science.

Title Efficient Visible/NIR Light-driven Uncaging of Hydroxylated Thiazole Orange-based Caged Compounds in Aqueous Media

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General

All reagents were purchased from Tokyo Chemical Industries, Wako Pure Chemical, or Sigma-Aldrich Chemical Co. and were used without further purification. Analytical thin-layer chromatography was performed on 60F254 silica plates (Merck & Co., Inc.) and visualized under UV light. Flash auto purification was conducted using the Isolera Spectra (Biotage) employing ZIP sphere, SNAP Ultra, and SNAP C18 cartridges. NMR spectra were recorded using an AVANCE500HD instrument (Bruker), with ^{1}H NMR spectra recorded at 500 MHz and ^{13}C NMR spectra recorded at 125 MHz using tetramethylsilane as an internal standard. FAB mass spectra were acquired using a JMS-700 mass spectrometer (JEOL), while electrospray ionization mass spectra were obtained using an LCT-Premier XE mass spectrometer (Waters). Reversed-phase HPLC analyses were performed using an Inertsil ODS-3 column (4.6 × 250 mm; GL Sciences, Inc.) and an HPLC system comprising a pump (PU-2080 [JASCO] or Chromaster® 5110 [HITACHI]) and detector (MD-2010 plus and FP-2020 [JASCO] or Chromaster® 5430 and Chromaster® 5440 [HITACHI]). The sample injections were performed using an autosampler (AS-4550 [JASCO] or Chromaster® 5210 [HITACHI]). Preparative HPLC separations were performed using an Inertsil ODS-3 column (10.0 × 250 mm; GL Sciences, Inc.) and an HPLC system comprising a pump (PU-2087; JASCO) and detector (UV-2075; JASCO). Buffer A was composed of 0.1% TFA in H_2O or 0.1% HCOOH in H_2O, and buffer B was composed of 0.1% TFA in acetonitrile or 0.1% HCOOH in acetonitrile.

UV-Vis absorption spectroscopy

The absorption spectra of HTO-OAc (10 μM) were measured in PBS buffer (pH 7.4) containing 10% DMSO at 25 ºC. The absorption spectra of HTO-OPip (10 μM) were measured in a 1:1 (v/v) PBS/CH_3CN mixture containing 0.3% DMSO at 25ºC (pH 7.4). The absorption spectra of Sul-HTO-Glu (10 μM) were measured in PBS (pH 7.4) containing 0.1% DMSO at 25 ºC.

One-photon uncaging and photochemical quantum yield determination

The photon flux of the lamp (*I*) was determined using ferrioxalate actinometry.[1] The solution contained 1.0 mL 0.1 M $K_3[Fe(C_2O_4)_3]\cdot 3H_2O$ in 0.1 N sulfuric acid and was prepared in a dark room using a red light. Irradiation was conducted using a Xe lamp (λ_{ex} = 490 ± 5 nm). Samples in HPLC vials were irradiated for 0, 10, 20, or 30 s, and 0.250 mL of each sample was immediately transferred to a 10-mL volumetric flask, followed by the addition of 0.5 mL buffer stock solution (8.2 g NaOAc, 1 mL conc. H_2SO_4, 100 mL water), 4 mL 0.1% 1,10-phenanthroline solution in water, and water to a final volume of 10 mL. After at least 1 h in the dark, 0.2 mL of each sample was transferred to a cuvette, and the absorbance was measured at 510 nm. The photon flux (I einstein·s^{-1}·cm^{-2}) was determined using the following equations:

$$I = \frac{\Delta n}{\Phi_\lambda \cdot S \cdot t} \cdot \frac{1}{1 - 10^{-A'}} \quad \text{(Eq. 1)}$$

$$\Delta n = \frac{V_1 \cdot V_3 \cdot 10^{-3}}{V_2} \cdot \frac{\Delta A}{\varepsilon_{510} \cdot l} \quad \text{(Eq. 2)}$$

I : Photon flux (einstein·s^{-1}·cm^{-2})
Δn : Fe^{2+} photogenerated (mol)
Φ_λ : Photochemical quantum yield of the actinometer at a given wavelength
S : Irradiated area (cm^2)
t : Irradiation time (s)
A' : Absorbance of the actinometer at the irradiation wavelength
V_1 : Volume of the irradiated sample (mL)
V_2 : Volume of the transferred irradiated sample (mL)
V_3 : Volume of the volumetric flask containing the buffer and phenanthroline (mL)
ΔA : Difference in absorbance from the 0 s (dark) experiment
ε_{510} : Absorption coefficient of the complex (11100 L mol^{-1} cm^{-1})
l : Optical pathlength of the cuvette (cm)

The photochemical quantum yields were obtained using HPLC time-course experiments. HTO compounds and internal standards were irradiated using a Xe lamp (light intensity: 10 mW/cm^2, λ_{ex} = 490 ± 5 nm) in solution (Table S1) at room temperature. HPLC analyses were performed using the Chromaster® system after injection of each sample (100 μL) using an autosampler. Relative peak areas were obtained by dividing the HTO compound peak areas by the internal standard peak area. The degradation rates of HTO compounds were determined by fitting a monoexponential decay curve. Quantum yields for photolysis were determined using the following equation (Eq. 3):

$$\Phi = (I\sigma t_{90\%})^{-1} \quad \text{(Eq. 3)}$$

I is the photon flux in einstein·s^{-1}·cm^{-2}, σ is the decadic extinction coefficient (10^3 times the molar extinction coefficient, ε) at cm^2·mol^{-1}, and $t_{90\%}$ is the irradiation time in seconds for 90% photoconversion to the product. The photon flux of the lamp I (2.9126×10^{-8} einstein·s^{-1}·cm^{-2}) was measured by potassium ferrioxalate actinometry (see the previous section for details).

Table S1. Solvent conditions and internal standards.

Compounds	Solvent	Internal standard
HTO-OAc	PBS buffer (10% DMSO, pH 7.4)	methyl 3-hydroxy-2-naphthoate
HTO-OPip	PBS buffer/acetonitrile = 1:1(3% DMSO, pH 7.4)	methyl 3-hydroxy-2-naphthoate
Sul-HTO-Glu	PBS buffer (3% DMSO, pH 7.4)	benzoic acid

Two-photon excitation

Sample solutions (15 µL) containing 50 µM Sul-HTO-Glu and 300 µM benzoic acid as internal standards were placed on a 35-mm glass bottomed dish (IWAKI), the surface of which contained fluorescent beads (FluoSpheres™ Polystyrene Microspheres 1.0 µm, orange fluorescence (ex/em = 540/560), Invitrogen, or Fluoresbrite® YG Microspheres, Calibration Grade 0.5 µm (ex/em = 441/486), Polysciences) as a reference point for setting the position of the two-photon focal volume within a droplet of sample solution. Samples were irradiated for 1 h with 940 nm (Sul-HTO-Glu) or 740 nm (MNI-Glu) light using a femtosecond-pulsed and mode-locked Ti:sapphire laser (Mai Tai DeepSee, Spectra-Physics) focused on the center of the droplet using an upright microscope (A1R-MP$^+$; Nikon) equipped with a water immersion objective lens (Apo LWD W, 1.10 NA, 25×, Nikon). The average power used was 150 mW at 940 nm and 200 mW at 740 nm, which was measured after passing through the objective lens using a power meter (PM100D; Thorlabs) and microscope power sensor head (S170C; Thorlabs). After irradiation, samples were analyzed by HPLC (Prominence series; Shimazu) analysis using a reversed-phase column (Poroshell 120 EC-C18, 4.6 × 100 mm; Agilent) to quantify the percentage of remaining starting material.

NMR photolysis experiments

HTO-OAc was dissolved in a 7:3 (v/v) mixture of DMSO-*d6* and D_2O. The sample was transferred into an NMR tube and irradiated with an LED light source (CL-1501; Asahi, light intensity: 100 mW/cm^2, λ_{ex} = 505 ± 5 nm,) for the time indicated. After irradiation, the ^{1}H NMR spectrum was recorded at 25 ºC in a 500 MHz spectrometer.

Oxygen-18 labeling experiments

As a control, a 100 µM solution of HTO-OAc in water containing 10% DMSO was irradiated using a Xe lamp (light intensity = 10 mW/cm^2, λ_{ex} = 490 ± 5 nm) while stirring at room temperature. The reaction was monitored by HPLC (Column: Inertsil® ODS3, buffer A: 0.1% HCOOH in H_2O, buffer B: 0.1% HCOOH in CH_3CN, buffer A/B ratio (%): 80/20 (0 min), 50/50 (30 min).) The fraction corresponding to HTO-OH was collected and analyzed using a mass spectrometer. A 100 µM solution of HTO-OAc in ^{18}O-labeled water containing 10% DMSO was irradiated using a Xe lamp (light intensity = 10 mW/cm^2, λ_{ex} = 490 ± 5 nm) while stirring at room temperature. The reaction was analyzed by HPLC and MS along with an unlabeled control experiment.

Quantification of glutamate

Glutamate in the aqueous solution was quantified using a fluorescent derivatization method with 4-fluoro-7-nitro-2,1,3-benzoxadiazole.[2] The derivatized fluorescent product was quantified by HPLC with fluorescence detection (ex/em = 470/530 nm). Buffer A contained 0.1% HCOOH in H_2O and buffer B was composed of 0.1% HCOOH in acetonitrile. Standard glutamate solutions (10, 20, 30, 40 µM) were used to prepare a calibration curve.

Plasmids

NMDA receptors plasmids were acquired from Addgene (pCI-EGFP-NR1 wt (#45446) and pCI-EGFP-NR2a wt (#45445)).[3] For oocyte experiments, untagged NR1 and NR2a were synthesized by removing GFP from GFP-NR1 and GFP-NR2 constructs.

Preparation of oocytes

Oocytes were prepared as described previously.[4] Briefly, *Xenopus* oocytes were collected from frogs anaesthetized in pure water containing 0.2% ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich, USA). The oocytes were defolliculated using type I collagenase (1.0 mg/mL, Sigma-Aldrich, USA) before cRNA injection. The plasmids were linearized by Not I and the cRNA was synthesized using a mMESSAGE mMACHINE T7 transcription kit (Thermo Fisher Scientific). A volume of 50 nL containing an equal amount of NR1 and NR2a cRNA was injected into each oocyte. The injected oocytes were incubated for 2–3 days at 18 °C in ND96 solution (5 mM HEPES, 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.5)).

Uncaging glutamate in oocytes

Currents were recorded using a two-electrode voltage clamp with an Oocyte Clamp OC-725C amplifier (Warner Instruments) and digitized by Digidata 1550B (Molecular Devices). Electrodes were filled with a 3 M KCl solution. The software for the acquisition and analysis of data was Axon™ pCLAMP™ (Molecular Devices). The bath solution contained 115 mM NaCl, 5 mM KCl, 10 mM HEPES, 1.8 mM BaCl2, and 0.01 mM EDTA (pH 7.3). The oocytes were irradiated with an LED light source (CL-1501; Asahi, λ_{ex} = 505 nm, laser intensity = 30 mW/cm^2, irradiation period = 10 s).

Cell culture and expression of NMDARs

HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and two antibiotics (100 units/mL penicillin and 0.1 mg/mL streptomycin). HEK293 cells expressing GFP-NR1 and GFP-NR2A subunits (1:1 ratio) were placed in a 35 mm glass-bottomed dish. Transient transfection was performed using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. At 4 h after transfection, the NMDAR antagonists DL-2-amino-5-phosphonovalerate (200 μM) and 7-chlorokynurenic acid (200 μM) were added to the culture medium to reduce the cytotoxic effects of NMDAR expression. Cells were evaluated at 24–48 h post-transfection.

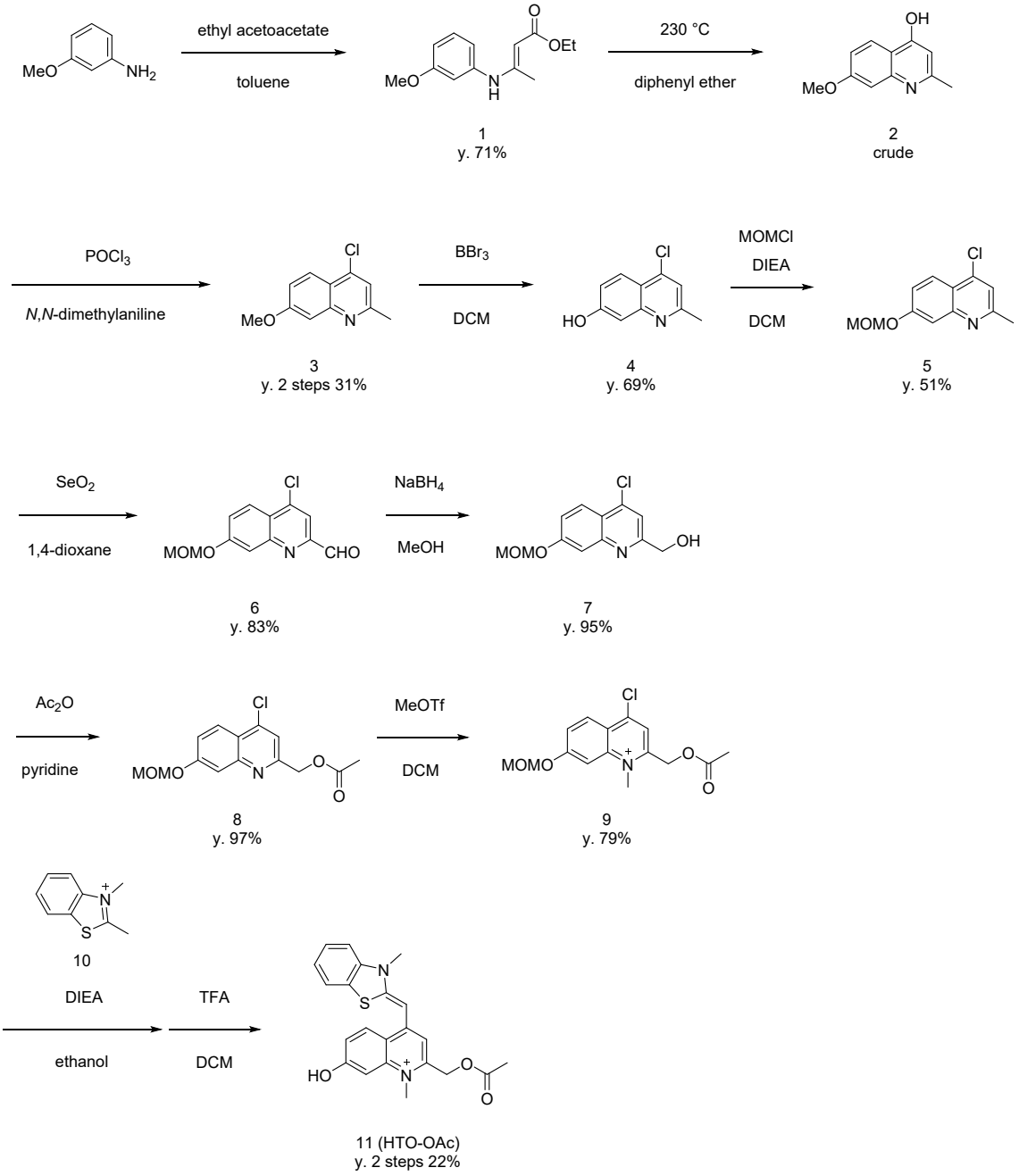
Calcium imaging

Calcium imaging was performed using an Olympus Fv10i confocal microscope equipped with a 60X lens. Non-transfected and transfected HEK293 cells were incubated in Mg^{2+}- and Ca^{2+}-free HEPES-buffered Hanks balanced salt solution (HHBSS) with 0.01% Pluronic F-127 (AnaSpec) containing 5 μg/mL Calbryte 590 AM Ca^{2+}-sensitive dye (AAT Bioquest) for 30 min at 37°C. The cells were rinsed twice with Mg^{2+}- and Ca^{2+}-free HHBSS before adding 10 mM Ca^{2+} in Mg^{2+}-free HHBSS containing 25 μM Sul-HTO-Glu and 50 μM glycine. Before the uncaging experiments, GFP fluorescence was used to localize GFP-NMDARs using the following excitation and emission wavelengths: GFP: 473 nm and 490–540 nm, respectively. Samples were uncaged with an LED light source (CL-1501; Asahi, λ_{ex} = 505 nm, laser intensity = 90 mW/cm^2, irradiation period = 10 s). Images were acquired every 5 s using the following excitation and emission wavelengths: Calbryte 590 AM: 559 nm and 570–620 nm, respectively. ImageJ was used for image analysis and presentation. The mean value of fluorescence in a cell was obtained by enclosing the cell in an ROI. $\Delta F/F_0$ was reported as the change in fluorescence intensity ($\Delta F = F_t - F_0$) relative to the fluorescence intensity which was calculated by averaging the frames (F_0) before irradiation.

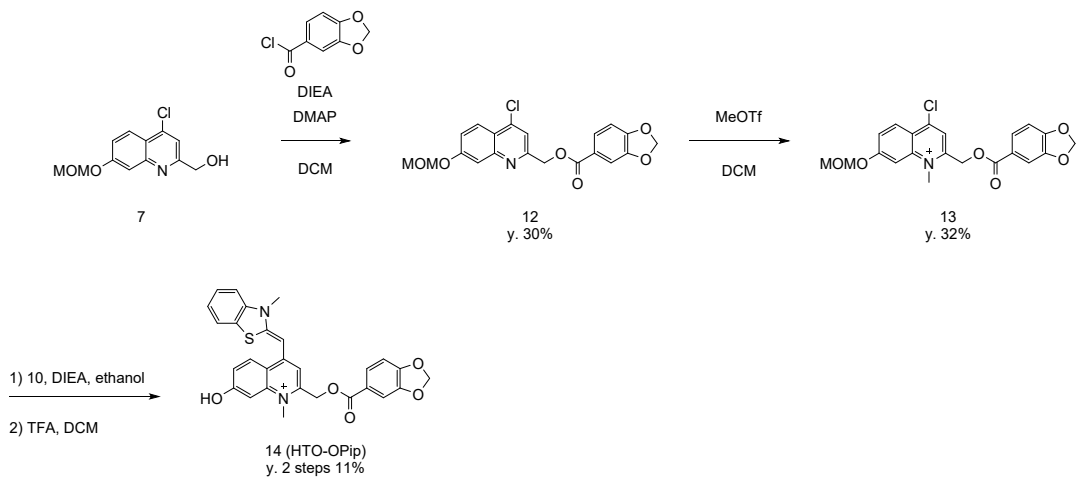
Cytotoxicity assay

HEK293T cells were re-plated into 96-well plates (1×10^4 cells/well) in DMEM containing 10% FBS and incubated overnight. The cells were then treated with the indicated concentrations of Sul-HTO-Glu. After 1 h, the cells were irradiated with an LED light source (CL-1501; Asahi, λ_{ex} = 505 nm, laser intensity = 30 or 90 mW/cm^2, irradiation period = 10 s). After 24 h, PrestoBlue™ cell viability reagent was added, and the cells were further incubated according to the manufacturer's guidelines. Cell viability was calculated by measuring the absorbance at 570 nm (experimental wavelength) and 600 nm (reference wavelength) using an M1000 microplate reader (Tecan).

Scheme S1. Synthesis of HTO-OAc.

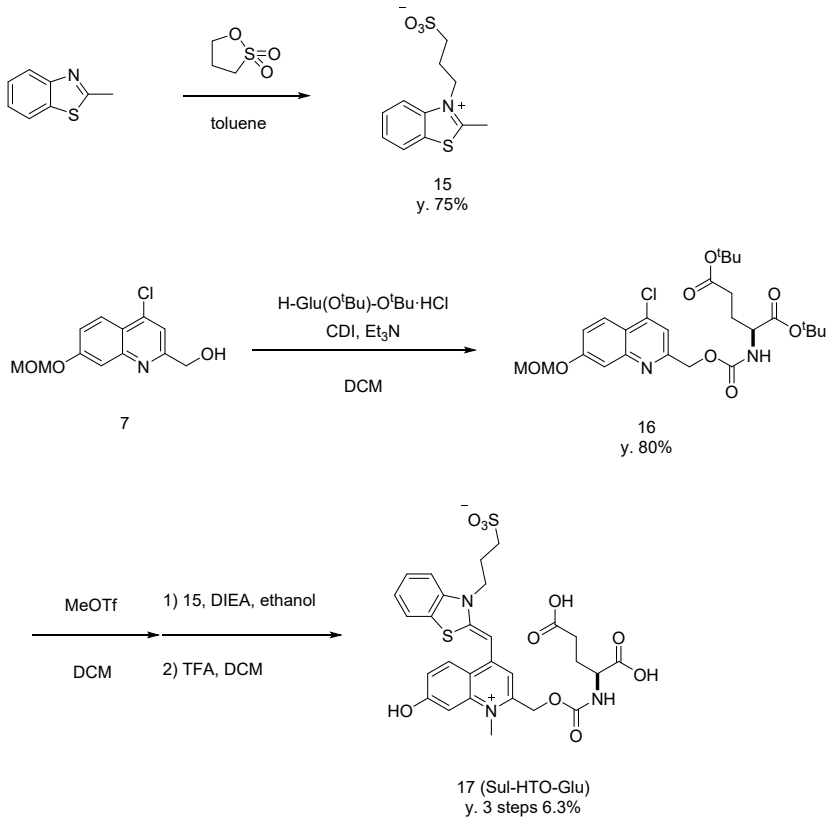


Scheme S2. Synthesis of HTO-OPip.



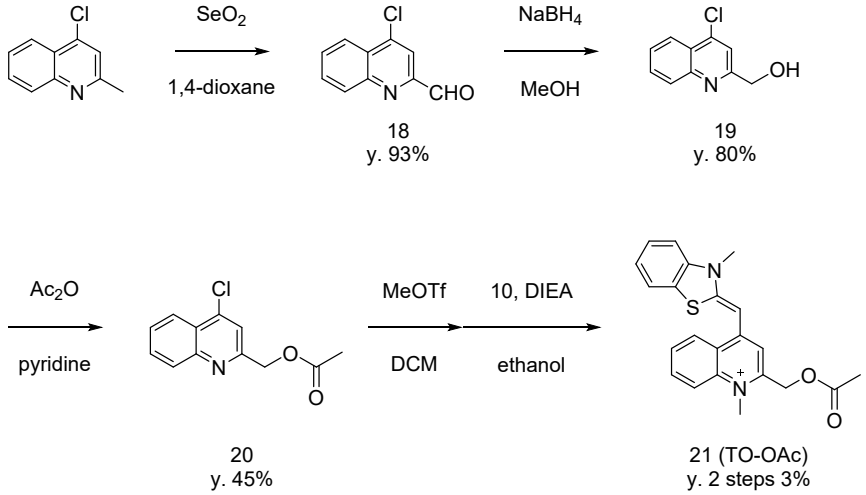
7 | 12 y. 30% | 13 y. 32% | 14 (HTO-OPip) y. 2 steps 11%

Scheme S3. Synthesis of Sul-HTO-Glu.



15 y. 75% | 7 | 16 y. 80% | 17 (Sul-HTO-Glu) y. 3 steps 6.3%

Scheme S4. Synthesis of TO-OAc.



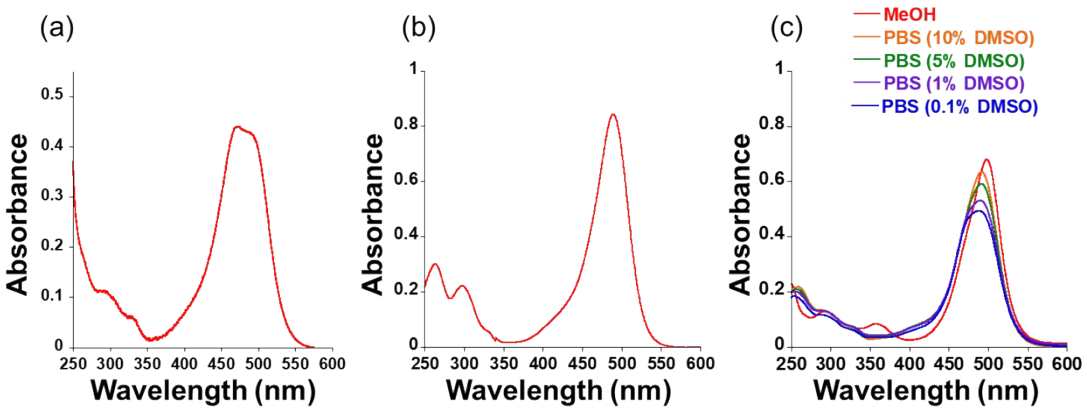


Figure S1. (a) Absorption spectra of HTO-Glu (10 μM) at 25 ºC in PBS buffer containing 10% DMSO (pH 7.4). (b) Absorption spectra of HTO-OPip (10 μM) at 25 ºC in PBS buffer: acetonitrile (1:1) with 0.3% DMSO (pH 7.4). (c) Absorption spectra of Sul-HTO-Glu (10 μM) at 25 ºC in PBS buffer containing 0.1–10% DMSO or methanol containing 0.1% DMSO (pH 7.4).

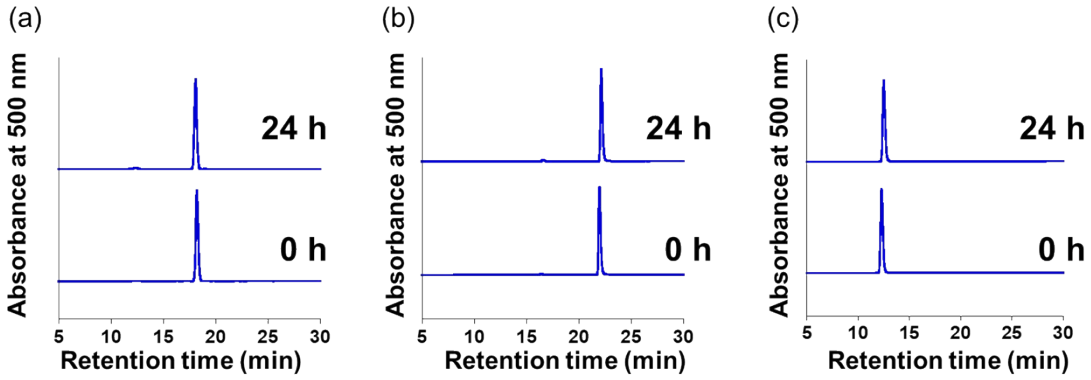


Figure S2. The stability of the caged HTO solutions kept in the dark. No changes were found in the chromatograms recorded after 24 h. (a) HTO-OAc (50 μM) in PBS containing 10% DMSO (pH 7.4), Buffer A: 0.1% TFA in H_2O, Buffer B: 0.1% TFA in CH_3CN, Buffer A/B ratio (%): 75/25 (0 min), 30/70 (30 min). (b) HTO-OPip (50 μM) in PBS buffer:acetonitrile (1:1) containing 3% DMSO (pH 7.4), buffer A: 0.1% HCOOH in H_2O, buffer B: 0.1% HCOOH in CH_3CN, buffer A/B ratio (%): 75/25 (0 min), 30/70 (30 min). (c) Sul-HTO-Glu (50 μM) in PBS containing 0.1% DMSO (pH 7.4), buffer A: 0.1% TFA in H_2O, buffer B: 0.1% TFA in CH_3CN, buffer A/B ratio (%): 80/20 (0 min), 50/50 (30 min).

	1	2	3	4	5	6
HTO cages	○	○		○	○	
Internal standard	○		○	○		
Light	○	○	○			○

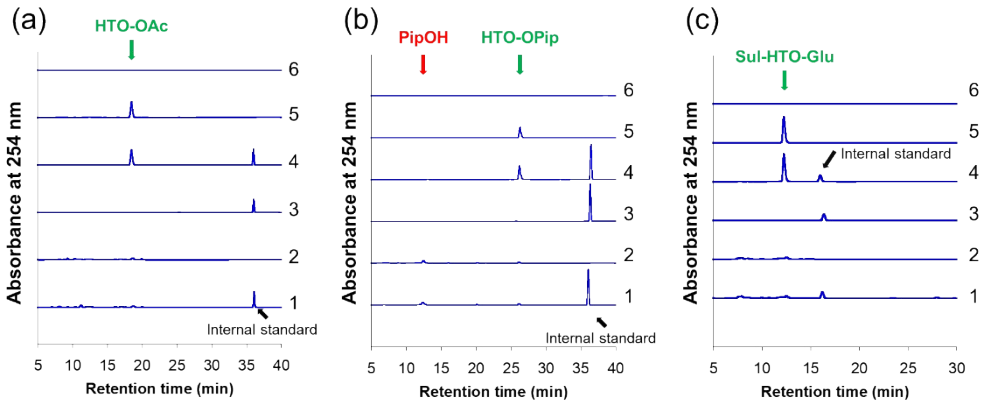


Figure S3. The effect of internal standards and solvents (Table S1) on the photolysis of HTO cages. Internal standards did not affect photolysis. (a) HTO-OAc (100 μM). (b) HTO-OPip (50 μM). (c) Sul-HTO-Glu (50 μM). Light = 10 mW/cm^2; λ = 490 ± 5 nm; (a) for 180 s, (b) for 20 min, (c) for 300 s.

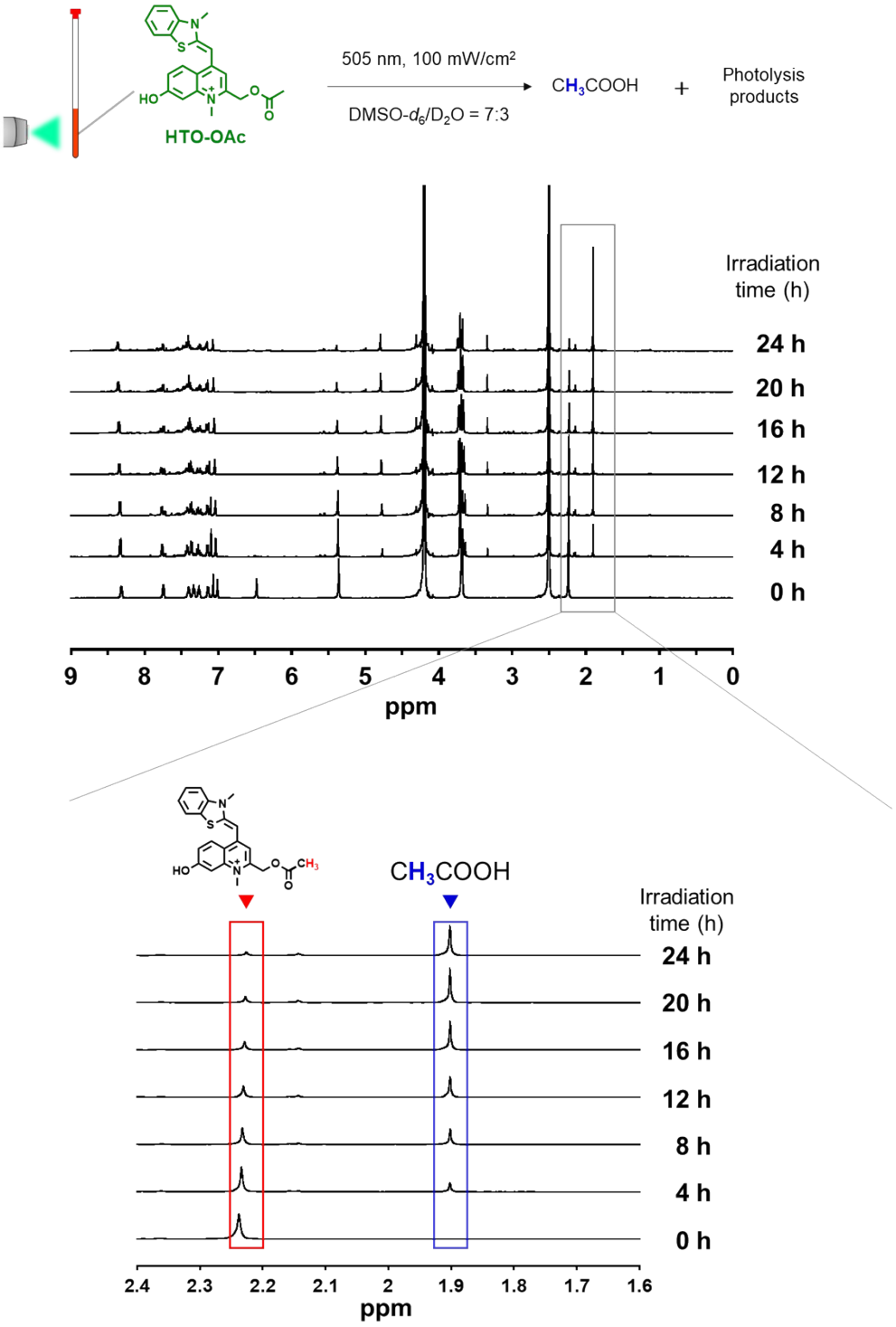


Figure S4. ^{1}H NMR spectrum of HTO-OAc after illumination with light (505 nm). Photo-induced release of acetic acid from HTO-OAc over time (bottom).

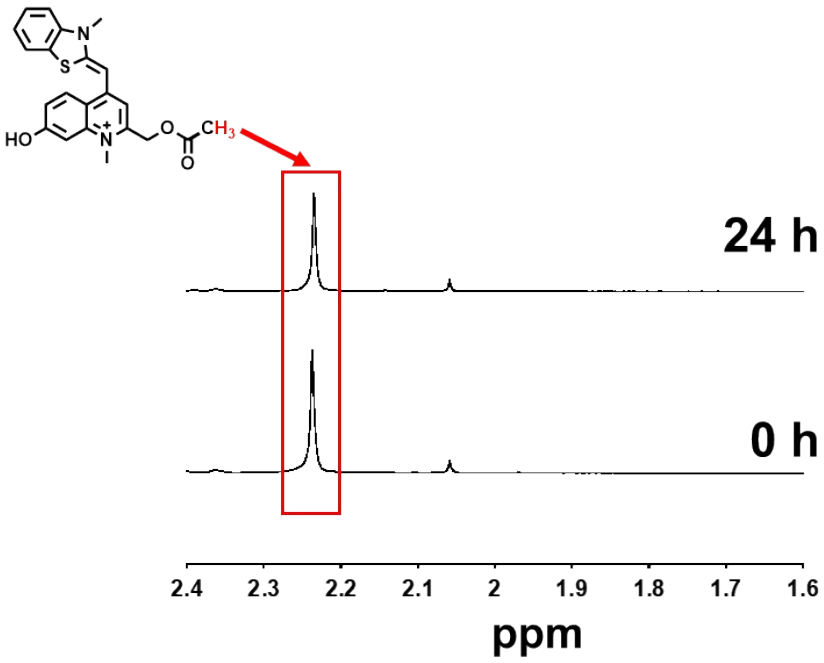


Figure S5. Stability of HTO-OAc in an NMR tube after 24 h in the dark.

Investigation of the photolysis reaction mechanism (Figure S6-S8)

The mechanism of the photolysis reaction of HTO was investigated. The photodegradation of BHQ PPG involves deprotonation of the 7-hydroxy group in the triplet- or singlet-excited state and leads to heterolysis of the C−O bond to generate an ion pair that subsequently collapses into the free leaving group and a solvent-captured side product.[5,6] This deprotonation proceeds in aqueous buffer rather than aprotic polar solvents. Thus, we compared the photolysis of Sul-HTO-Glu in buffer containing polar aprotic acetonitrile solvent by monitoring the absorption. Under illumination, we found a decrease in the absorption of Sul-HTO-Glu at around 490 nm in PBS buffer, corresponding with the results of HPLC analysis (Figure 3c). In contrast, we found that photolysis of Sul-HTO-Glu was significantly reduced in the presence of 50% acetonitrile in PBS buffer (Figure S6a), indicating that deprotonation is involved in HTO photodegradation. Moreover, there were no significant differences in the photodegradation kinetics between non-degassed and degassed samples (Figure S6 b-d), indicating that molecular oxygen did not influence the HTO photochemical reaction, unlike cyanine (Cy7)-based PPGs.[7]

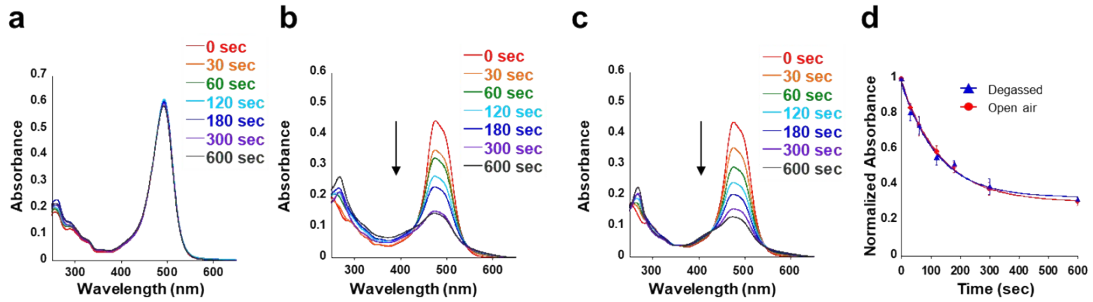


Figure S6. Absorption spectra of 10 μM Sul-HTO-Glu at 25 ºC after exposure to 490 nm light. Light intensity = 10 mW/cm², λ = 490 ± 5 nm. (a) In 1:1 PBS buffer/MeCN containing 0.1% DMSO (pH 7.4). (b) Degassed solution (pH 7.4 PBS buffer containing 0.1% DMSO). The samples were degassed using a freeze-pump-thaw technique (three cycles). (c) Open air solution (pH 7.4 PBS buffer containing 0.1% DMSO). (d) Absorbance traces at 490 nm. Blue: degassed solution. Red: open air solution. Data are presented as means ± SD (*N* = 3).

To further examine the role of the hydroxy group in the HTO scaffold, we synthesized TO-OAc, which lacks a hydroxy group in the quinoline ring (Scheme S4). Illumination of TO-OAc led to reduced consumption of TO-OAc versus HTO-OAc (Figure S7). This result indicates that the hydroxy group in HTO plays an important role in photochemical efficiency, as well as with BHQ and *N*-me-7HQm PPGs.

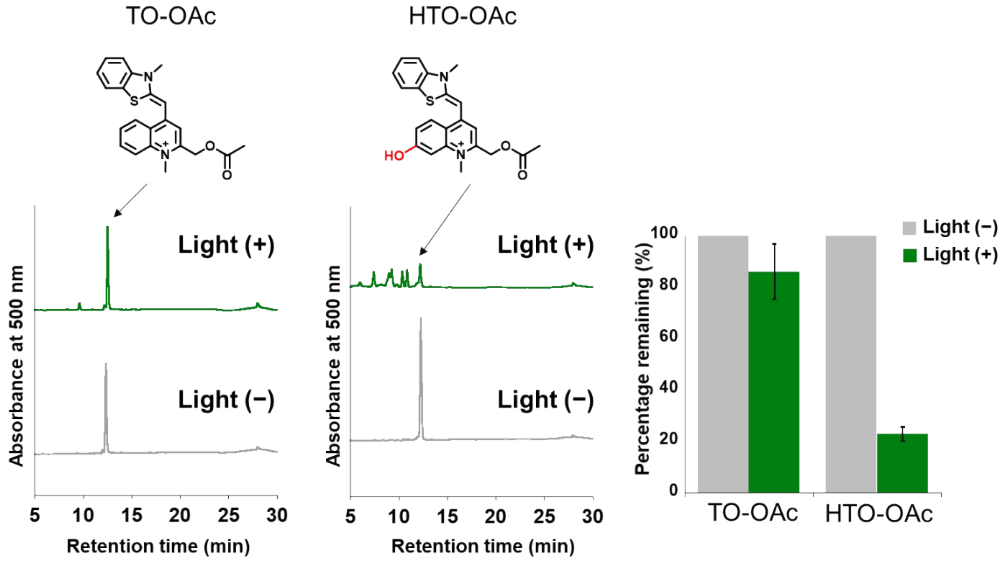


Figure S7. Illumination of TO-OAc and HTO-OAc. Column: Inertsil® ODS3, buffer A: 0.1% HCOOH in H_2O, buffer B: 0.1% HCOOH in CH_3CN, buffer A/B ratio (%): 75/25 (0 min), 30/70 (30 min). Light: λ = 490 ± 5 nm, 10 mW/cm², 120 s. Data are presented as means ± SD (*N* = 3).

In addition, we detected the photocleavage product containing ^{18}O (HTO-^{18}OH, *m/z* 353) in the product mixtures after photolysis of HTO-OAc in ^{18}O-labeled water (Figure S8). These results indicate that photolysis generated a solvent-captured side product via heterolysis of the C−O bond. From these results, we inferred that photodegradation of HTO resulted from the same photolysis mechanism as that with quinoline/quinolinium-based PPGs.

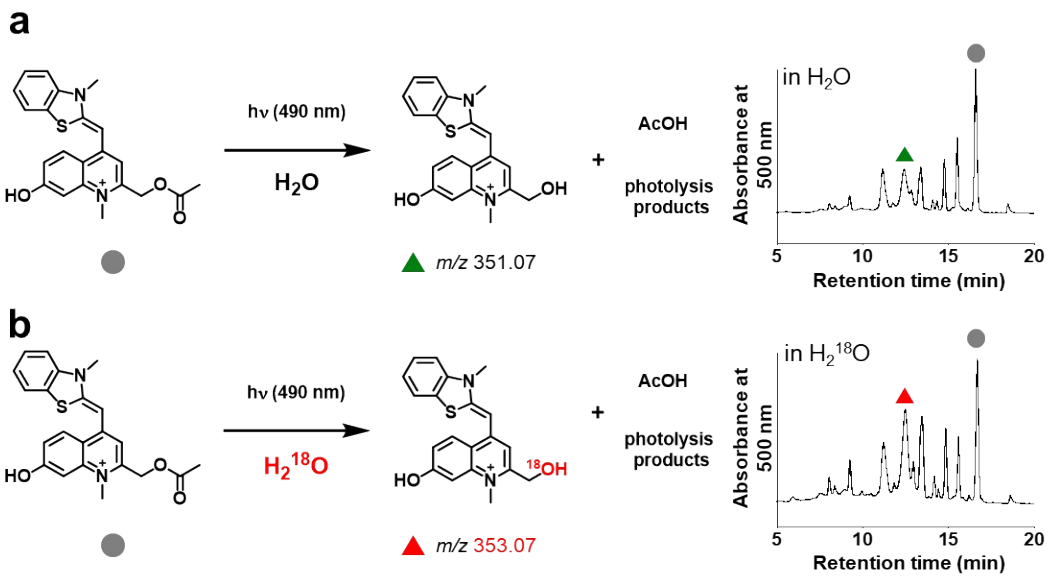


Figure S8. Oxygen-18 labeling experiment through photolysis of 100 µM HTO-OAc in (a) H_2O containing 10% DMSO and in (b) $H_2{}^{18}O$ (97% labelled) containing 10% DMSO. Light intensity = 10 mW/cm², λ = 490 ± 5 nm.

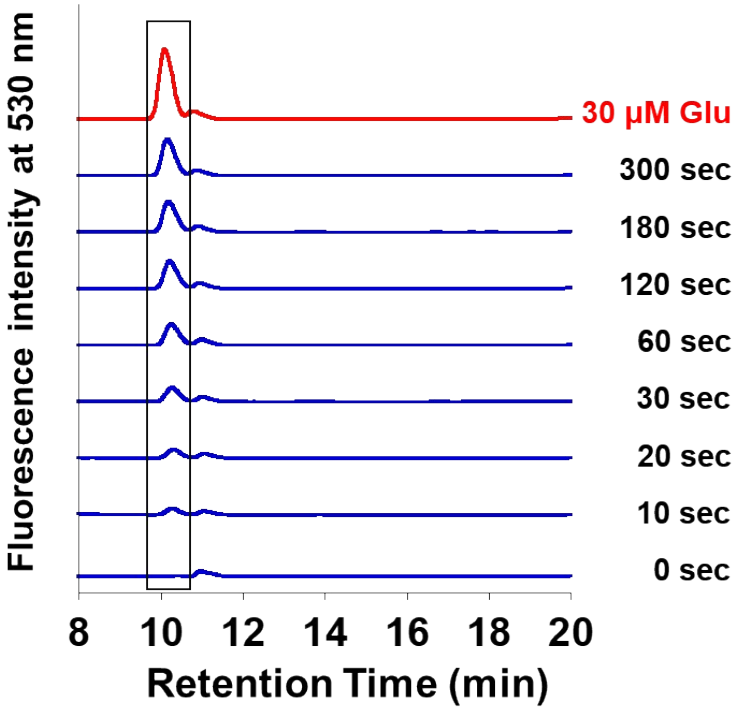


Figure S9. Time course of glutamate production using 50 µM Sul-HTO-Glu. Glutamate was derivatized to a fluorescent product using a fluorescence derivatization reagent (4-fluoro-7-nitro-2,1,3-benzoxadiazole). Column: Inertsil® ODS3; buffer A: 0.1% HCOOH in H_2O, buffer B: 0.1% HCOOH in CH_3CN, buffer A/B ratio (%): 75/25 (0 min), 30/70 (30 min). λ_{ex} = 470 nm, λ_{em} = 530 nm.

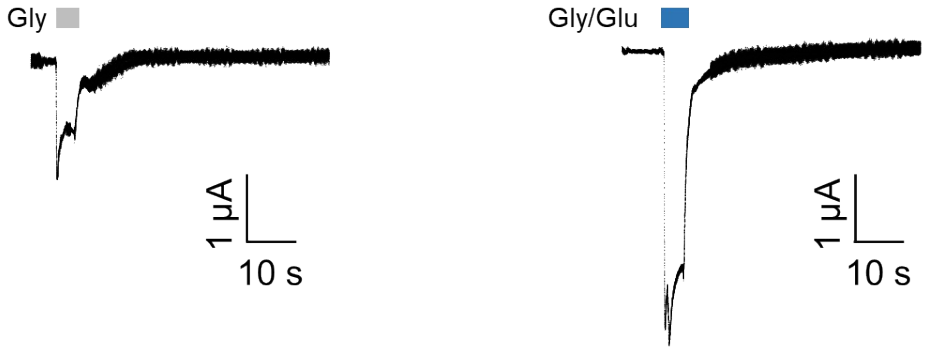


Figure S10. Oocyte membrane current response to 10 μM glycine (left) and 100 μM glutamate/10 μM glycine (right) treatments in a bath solution (pH 7.3).

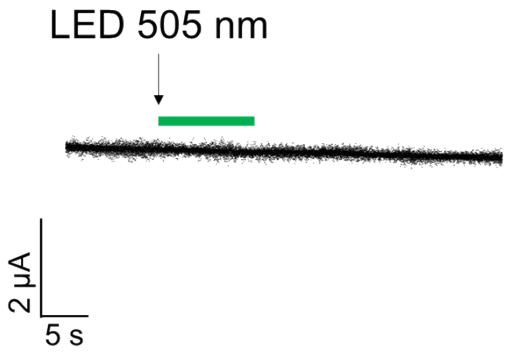


Figure S11. Oocyte membrane current in the absence of SuI-HTO-Glu and glycine. Light intensity = 30 mW/cm^2, λ = 505 nm. Light irradiation for 10 s.

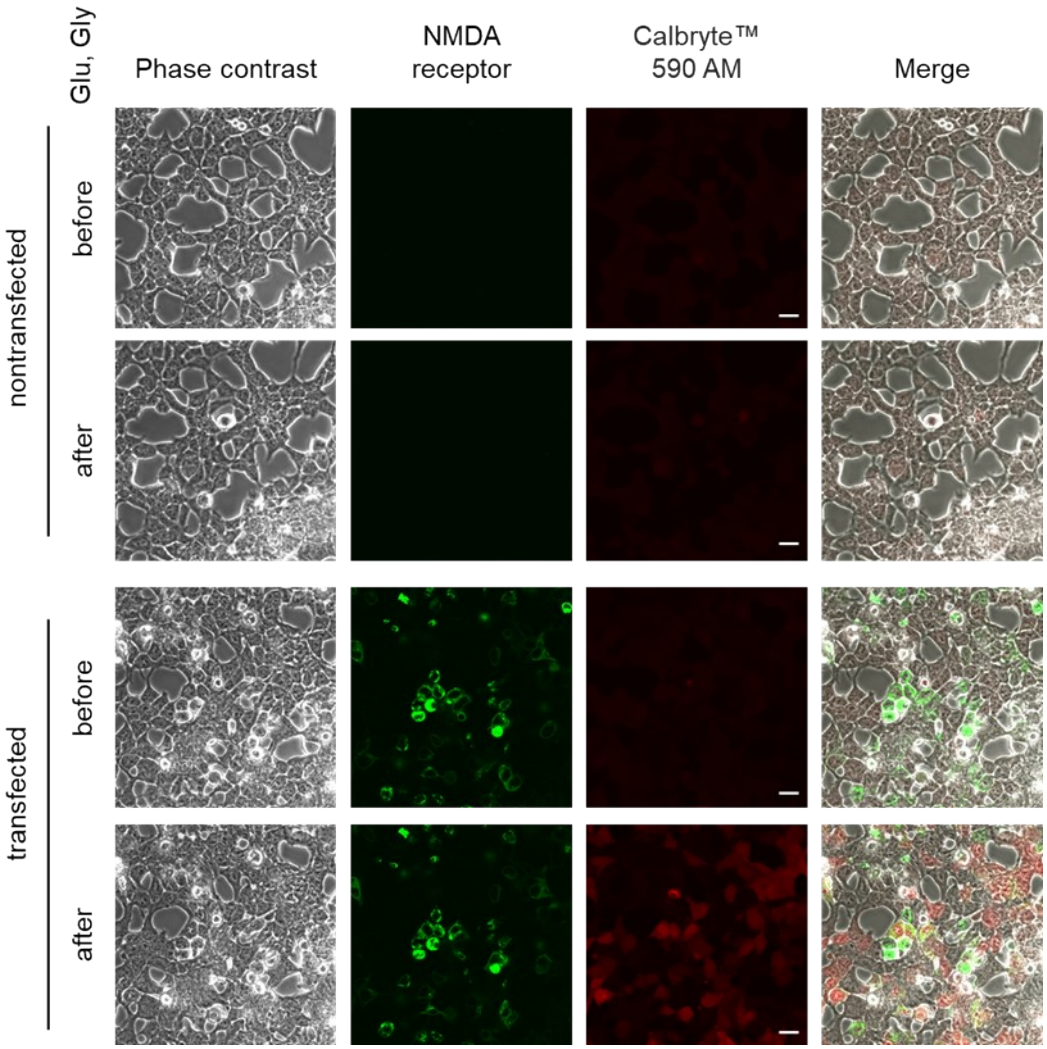


Figure S12. Non-transfected or transfected HEK293 cells loaded with 5 μg/mL Calbryte 590 AM Ca^{2+}-sensitive dye and stimulated with 10 μM glutamate and glycine. Scale bar: 20 μm.

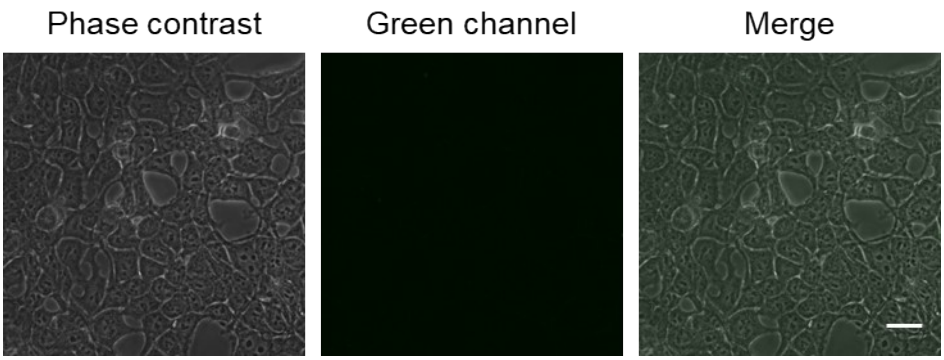


Figure S13. Confocal images of Sul-HTO-Glu (25 μM) with 473 nm excitation in HEK293T cells. Scale bar: 20 μm. Imaging condition was same with Figure S12.

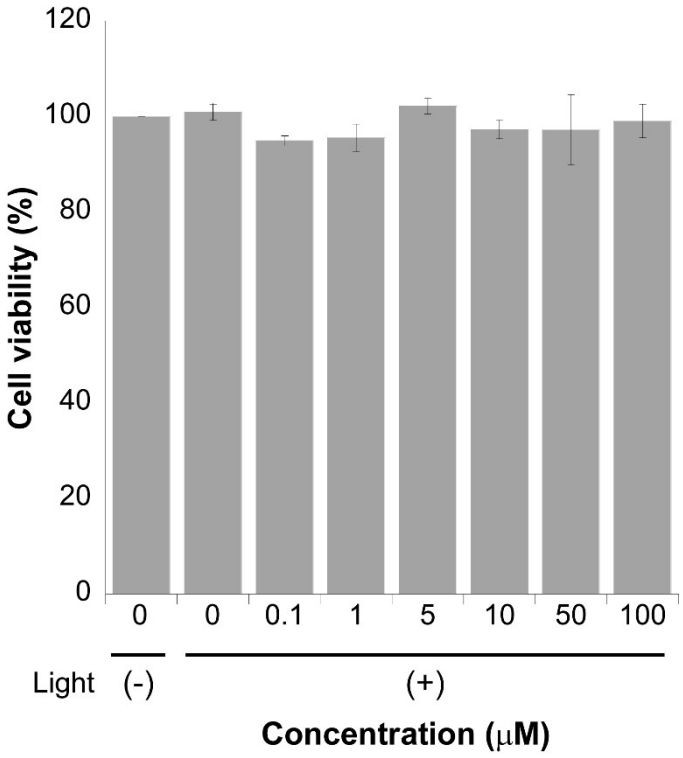


Figure S14. Cytotoxicity results for Sul-HTO-Glu at varying concentrations (0–100 μM) in HEK293T cells. Light = 30 mW/cm^2, Illumination period = 10 s. Error bars represent the SD (*N* = 3).

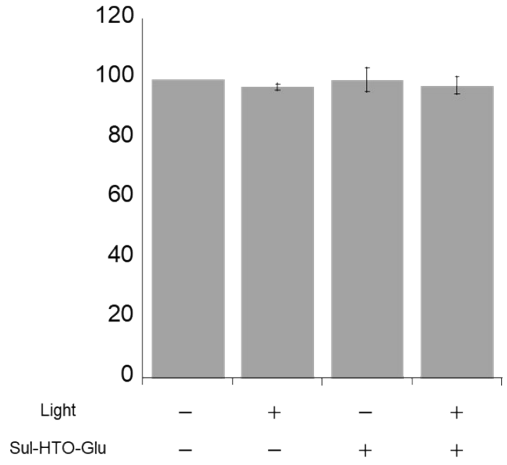
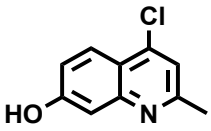


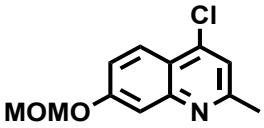
Figure S15. Cytotoxicity results of Sul-HTO-Glu (25 μM) under Ca^{2+} imaging conditions in HEK293T cells. Light = 90 mW/cm^2, Illumination period = 10 s. Error bars represent the SD (*N* = 3).

Synthesis of compounds

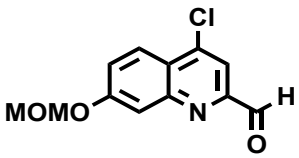
Compound 1, 2, 3
Compound 1,2,3 were prepared according to literature procedures[7].



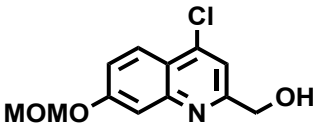
4-chloro-2-methylquinolin-7-ol. (compound 4)
Under a nitrogen atmosphere, BBr_3 (1 M solution in DCM, 4.66 mL, 4.66 mmol) was added to a solution of compound 3 (363 mg, 1.75 mmol) in DCM 15 mL at 0 °C. After complete addition, the ice bath was removed and stirring was continued for 3 h at room temperature. The reaction mixture was then added dropwise to a mixture of water and ice (30 mL) under stirring. After complete addition, the mixture was stirred for 30 min at room temperature and then filtered to give, after drying 4-chloro-2-methylquinolin-7-ol as a yellow solid (255 mg, 0.753 mmol, 75%): ^{1}H NMR (500 MHz, MeOD) δ 8.37 (d, *J* = 9.0 Hz, 1H), 7.85 (s, 1H), 7.52 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 2.89 (s, 3H); ^{13}C NMR (125 MHz, MeOD) δ 164.8, 156.7, 151.9, 141.3, 127.3, 122.6, 120.0, 119.9, 101.5, 19.9; HRMS (FAB+) Calcd for $[M+H]^+$, 194.0372, found for 194.0377.



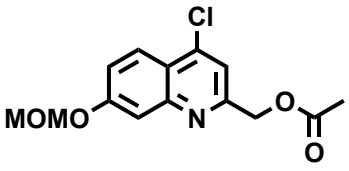
4-chloro-7-(methoxymethoxy)-2-methylquinoline. (compound 5)
Under a nitrogen atmosphere, *N*,*N*-diisopropylethylamine (0.225 mL, 1.30 mmol) was added to the solution of compound 4 (100 mg, 0.518 mmol) in dry DMF (10 mL). chloromethyl methyl ether (0.098 mL, 1.30 mmol) was added to the reaction mixture dropwise. After stirring for 2 h, The solvent was concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 EtOAc/hexane) to afford compound 5 as a colorless solid (71.7 mg, 0.302 mmol): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 9.0 Hz, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.29 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 7.27 (s, 1H), 5.32 (s, 2H), 3.51 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.5, 158.8, 150.2, 142.3, 125.2, 120.3, 120.1, 119.6, 111.0, 94.4, 56.3, 25.1; HRMS (FAB+) Calcd for $[M+H]^+$, 238.0635, found for 238.0638.



4-chloro-7-(methoxymethoxy)quinoline-2-carbaldehyde. (compound 6)
Under a nitrogen atmosphere, selenium dioxide (135 mg, 1.22 mmol) was added to a solution of compound 5 (262 mg, 1.11 mmol) in dioxane (30 mL). The reaction was stirred for 4 h at 85 °C, then cooled, diluted with methanol, and vacuum filtered. The filtrate was collected and concentrated, leaving a yellow solid, which was purified by column chromatography (2:8 EtOAc/hexane) to provide compound 6 (231 mg, 0.920 mmol) as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.14 (s, 1H) 8.22 (d, *J* = 9.0 Hz, 1H), 7.97 (s, 1H), 7.82 (d, *J* = 2.0 Hz, 1H), 7.50 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 5.38 (s, 2H), 3.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.7, 159.4, 152.8, 150.4, 144.0, 125.6, 123.7, 123.5, 116.0, 112.1, 94.5, 56.5; HRMS (FAB+) Calcd for $[M+H]^+$, 252.0427, found for 252.0426.

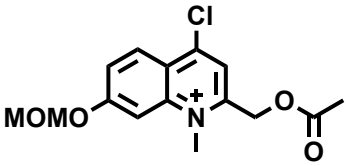


(4-chloro-7-(methoxymethoxy)quinolin-2-yl)methanol (compound 7)
$NaBH_4$ was added to a solution of compound 6 (70.0 mg, 0.279 mmol) in methanol (15 mL). The reaction mixture was stirred for 15 min, diluted with EtOAc, washed with water followed by brine. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to afford compound 7 (67.2 mg, 0.266 mmol) as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J* = 9.0 Hz, 1H), 7.65 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 7.28 (s, 1H), 5.38 (s, 2H), 4.86 (s, 2H), 4.15(br, 1H), 3.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.6, 159.0, 149.1,143.0, 125.5, 121.3, 120.2, 116.6, 111.0, 94.4, 63.9, 56.4; HRMS (FAB+) Calcd for $[M+H]^+$, 254.0584, found for 254.0578.



(4-chloro-7-(methoxymethoxy)quinolin-2-yl)methyl acetate (compound 8)
compound 7 (100 mg, 0.395 mmol) was dissolved in pyridine (2.5 mL). To the solution, acetic anhydride (37 µL, 0.395 mmol) was added. After being stirred at room temperature for 4 h, the reaction mixture was concentrated in vacuo, followed by flash

chromatography to afford compound 8 as a colorless solid (83.0 mg, 0.281 mmol): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, *J* = 9.0 Hz, 1H), 7.65 (d, *J* = 2.0 Hz, 1H), 7.43 (s, 1H), 7.35 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 5.33 (s, 2H), 5.32 (s, 2H), 3.52 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 159.0, 156.7, 150.1, 143.2, 125.3, 121.3, 120.8, 117.7, 111.2, 94.4, 66.9, 56.4, 20.9; HRMS (FAB+) Calcd for [M+H]+, 296.0690, found for 296.0691.

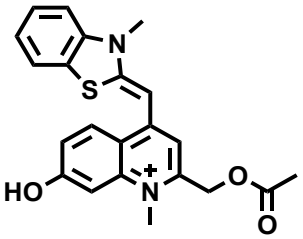


4-chloro-2-(hydroxymethyl)-7-(methoxymethoxy)-1-methylquinolin-1-ium (Compound 9)

To a solution of compound 8 (50 mg, 0.169 mmol) in DCM (1.0 mL) was added dropwise Methyl trifluoromethanesulfonate (37 µL, 0.339 mmol). After being stirred at room temperature for 4 h, the reaction mixture was concentrated in vacuo, followed by flash chromatography (10:1 DCM/MeOH) to provide the compound 9 (41.3 mg, 0.133 mmol) as a blue powder: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (d, *J* = 9.5 Hz, 1H), 7.91 (s, 1H), 7.80 (d, *J* = 2.0 Hz, 1H), 7.66 (dd, *J* = 9.5 Hz, *J* = 2.0 Hz, 1H), 5.72 (s, 2H), 5.51 (s, 2H), 4.47 (s, 3H), 3.55 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 164.2, 155.7, 152.9, 143.1, 128.5, 123.7, 122.9, 120.6, 102.2, 95.5, 62.4, 57.2, 40.1, 20.5; HRMS (FAB+) Calcd for [M+H]+, 310.0841, found for 310.0847

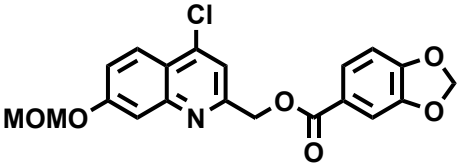
Compound 10

Compound 10 was prepared according to a literature procedure[8].



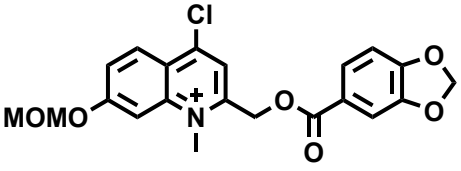
(*Z*)-2-(acetoxymethyl)-7-hydroxy-1-methyl-4-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)quinolin-1-ium (compound 11)

To a solution of the above compound 9 in ethanol (2.0 mL) was added compound 10 (42.6 mg, 0.258 mmol) and *N*,*N*-diisopropylethylamine (45 µL, 0.258 mmol). After being stirred at room temperature for 3 h in the dark, the solvent was removed under reduced pressure. The residue was used in the next step without further purification. To a solution of crude in DCM (4.00 mL) was added TFA (1.00 mL). After stirring for 2 h, a solvent was removed under reduced pressure. The residue was purified by reversed-phase HPLC under the following conditions: A/B = 80/20 (0 min), 50/50 (30 min), (solvent A: 0.1% HCOOH in H_2O; solvent B: 0.1% HCOOH in CH_3CN). After lyophilization, an orange powder of compound 11 (5.30 mg, 0.0135 mmol) was obtained: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.65 (d, *J* = 9.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.59 (t, *J* = 8.0 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.36 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H,) 7.26 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 6.82 (s, 1H), 5.54 (s, 2H), 3.96 (s, 3H), 3.91 (s, 3H), 2.29 (s, 3H) ; ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 162.9, 159.7, 149.7, 148.5, 142.1, 141.0, 128.6, 128.1, 124.7, 124.1, 123.3, 118.0, 117.3, 113.2, 107.4, 101.9, 88.1, 63.1, 37.0, 34.1, 21.2; HRMS (FAB+) Calcd for $[M]^+$ 393.1267, found for 393.1271



(4-chloro-7-(methoxymethoxy)quinolin-2-yl)methylbenzo[*d*][1,3]dioxole-5-carboxylate (compound 12)

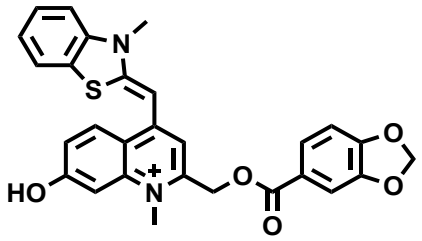
Under a nitrogen atmosphere, compound 7 (100 mg, 0.242 mmol) was dissolved in DCM (10 mL). *N*,*N*-diisopropylethylamine (45 µL, 0.36 mmol) and *N*,*N*-dimethyl-4-aminopyridine (10 mg, 0.08 mmol) were added, followed by piperonylic chloride (50 mg, 0.27 mmol). The reaction was stirred for 2 h. The mixture was washed with 0.1 M citric acid, water, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography (3:7 EtOAc/hexane) to afford compound 12 as a colorless solid (90 mg, 0.16 mmol): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, *J* = 9.0 Hz, 1H), 7.76 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H), 7.65 (d, *J* = 2.0 Hz, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.50 (s, 1H), 7.36 (dd *J* = 9.0 Hz, *J* = 2.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.06 (s, 2H), 5.54 (s, 2H), 5.34 (s, 2H), 3.52 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 159.0, 157.0, 152.0, 151.1, 147.9, 143.3, 125.8, 125.4, 123.6, 121.4, 120.8, 117.6, 111.2, 109.7, 108.1, 102.0, 94.4, 67.3, 56.4; HRMS (FAB+) Calcd for $[M+H]^+$, 402.0744, found for 402.0749



2-(((benzo[*d*][1,3]dioxole-5-carbonyl)oxy)methyl)-4-chloro-7-(methoxymethoxy)-1-methylquinolin-1-ium (compound 13)

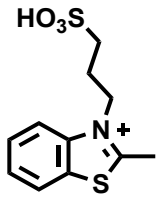
To a solution of compound 12 (33 mg, 0.823 mmol) in DCM (1.0 mL) was added dropwise Methyl trifluoromethanesulfonate (18 µL, 0.165 mmol). After being stirred at room temperature for 4 h, the reaction mixture was concentrated in vacuo, followed by flash chromatography (10:1 DCM/MeOH) to provide the compound 13 (16.0 mg, 0.321 mmol) as a blue powder: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, *J* = 9.0 Hz, 1H), 7.92 (s, 1H), 7.81 (d, *J* = 2.0 Hz, 1H), 7.73 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H), 7.66 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz,

1H), 7.48 (d, *J* = 2.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.09 (s, 2H), 5.96 (s, 2H), 5.50 (s, 2H), 4.57 (s, 3H), 3.56 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0, 164.2, 156.0, 153.0, 152.9, 148.2, 143.1, 128.6, 126.4, 123.6, 122.9, 121.6, 120.7, 109.6, 108.5, 102.2, 102.1, 95.4, 62.8, 57.2, 40.2; HRMS (FAB+) Calcd for $[M]^+$, 416.0895, found for 416.0900.



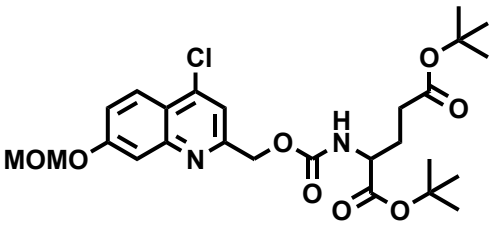
***(Z)*-2-(((benzo[*d*][1,3]dioxole-5-carbonyl)oxy)methyl)-7-hydroxy-1-methyl-4-((3-methylbenzo[*d*]thiazol-2(3H)-ylidene)methyl)quinolin-1-ium (compound 14)**

Compound 10 (13.2 mg, 0.0803 mmol) was added to the solution of compound 13 (16.7 mg, 0.0401 mmol) in ethanol (2 mL). *N*,*N*-diisopropylethylamine (0.014 mL, 0.0803 mmol) was added to the reaction mixture dropwise. After stirring at room temperature for 3 h, the solvent was concentrated under reduced pressure. The residue was purified by flash chromatography (20:1 DCM/MeOH) to provide the orange powder, which was used immediately in next step. To the solution of crude in DCM (5 mL) was added TFA (2 mL). After stirring for 2 h, a solvent was removed under reduced pressure. The residue was purified by reversed-phase HPLC under the following conditions: A/B = 35/65 (0 min), 25/75 (30 min), (solvent A: 0.1% HCOOH in H_2O; solvent B: 0.1% HCOOH in CH_3CN). After lyophilization, an orange powder of compound 14 (3.35 mg, 0.00285 mmol) was obtained. ^{1}H NMR (500 MHz, CD_3CN) δ 8.28 (d, *J* = 9.5 Hz, 1H), 7.75 (d, *J* =8.0 Hz, 1H), 7.56 (s, 1H), 7.45 (d, *J* =8.0 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 9.5 Hz, 1H), 7.34 (s, 1H), 7.29 (s, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.52 (s, 1H), 6.02 (s, 2H), 5.54 (s, 2H), 3.83 (s, 3H), 3.71 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD); δ 168.0, 165.5, 159.1, 153.4, 149.3, 148.7, 148.1, 143.2, 141.0, 128.6, 127.1, 126.8, 124.8, 124.2, 122.9, 122.4, 121.0, 117.2, 112.2, 110.0, 108.8, 107.6, 102.9, 101.7, 87.8, 63.1, 36.4, 33.4; HRMS (FAB+) Calcd for $[M]^+$, 499.1322, found for 499.1327.



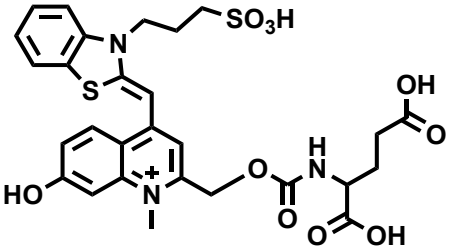
3-(2-methylbenzo[*d*]thiazol-3-ium-3-yl)propane-1-sulfonate (compound 15)

To a solution of 2-methylbenzothiazole (1000 mg, 6.06 mmol) in toluene (5.0 mL) was added 1,3-propanesultone (1110 mg, 9.09 mmol). The solution was refluxed for 24 hours. The precipitate produced was filtered under suction, washed with toluene and dried *in vacuo* to afford compound 15 (1367 mg, 5.04 mmol, 75%) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ 8.10 (d, *J* = 8.5 Hz, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 4.81 (m, 2H), 3.11 (s, 3H), 3.05 (t, *J* = 7.0 Hz, 2H), 2.34-2.28 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 176.3, 141.0, 129.8, 129.0, 128.5, 123.8, 116.2, 47.7, 47.4, 23.1, 16.2; HRMS (FAB+) Calcd for $[M]^+$, 272.0415, found for 272.0413.



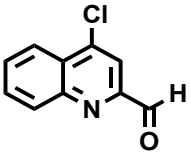
di-*tert*-butyl(((4-chloro-7-(methoxymethoxy)quinolin-2-yl)methoxy)carbonyl)glutamate (compound 16)

Under a nitrogen atmosphere, compound 7 (100 mg, 0.395 mmol) was dissolved in dry DCM (1.5 mL). 1,1'-Carbonyldiimidazole (769 mg, 0.474 mmol) was added, and the mixture was stirred at 0 ºC for 1 h. To the stirred solution were added H-Glu-(O^{t}Bu)-O^{t}Bu·HCl (233.8 mg, 0.790 mmol) and Et_3N (55.0 µL, 0.395 mmol) at 0 ºC. After stirring at room temperature for 23 h, the solvent was concentrated under reduced pressure. After being diluted with Et_2O, the mixture was washed with water, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography (3:7 EtOAc/hexane) to afford compound 16 (171 mg, 0.318 mmol, 80%) as a colorless solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, *J* = 9.0 Hz, 1H), 7.64 (d, *J* = 2.5 Hz, 1H), 7.46 (s, 1H), 7.34 (dd, *J* = 9.0 Hz, *J* = 2.5 Hz, 1H), 5.54 (d, *J* = 8.0 Hz, 1H) 5.33 (s, 2H), 5.32 (s, 2H), 4.32-4.28 (m, 1H), 3.51 (s, 3H), 2.41-2.27 (m, 2H), 2.20-2.13 (m, 1H), 1.98-1.91 (m, 1H), 1.48 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$); δ 171.1, 170.0, 158.9, 157.4, 155.6, 150.0, 143.2, 125.3, 121.3, 120.7, 117.5, 111.2, 94.4, 82.5, 80.8, 67.3, 56.3, 54.1, 28.1, 28.0, 27.9; HRMS (FAB+) Calcd for $[M]^+$, 539.2160, found for 539.2152.



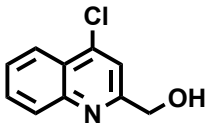
(*Z*)-3-(2-((2-((((1,3-dicarboxypropyl)carbamoyl)oxy)methyl)-7-hydroxy-1-methyl-1l4-quinolin-4-yl)methylene)benzo[*d*]thiazol-3(2H)-yl)propane-1-sulfonate (compound 17)

To a solution of compound 16 (60 mg, 0.111 mmol) in DCM (1.0 mL) was added dropwise methyl trifluoromethanesulfonate (24 µL, 0.223 mmol). After being stirred at room temperature for 4 h, the reaction mixture was concentrated in vacuo, which was used in the next step without further purification. To a solution of the above quinolinium in ethanol (2.5 mL) was added compound 15 (44 mg, 0.163 mmol) and *N*,*N*-diisopropylethylamine (28 µL, 0.163 mmol). After being stirred at room temperature for 3 h in the dark, the solvent was removed under reduced pressure. The residue was used in the next step without further purification. To a solution of crude in DCM (4.00 mL) was added TFA (1.00 mL). After stirring for 2 h, a solvent was removed under reduced pressure. The residue was purified by reversed-phase HPLC under the following conditions: A/B = 75/25 (0 min), 35/65 (30 min), (solvent A: 0.1% HCOOH in H_2O; solvent B: 0.1% HCOOH in CH_3CN). After lyophilization, an orange powder of compound 17 (3.24 mg, 0.00513 mmol) was obtained: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.1 (s, 1H), 8.94 (d, *J* = 9.5 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz 1H), 7.55 (t, *J* = 8.0 Hz 1H), 7.38 (s, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 2.0 Hz, 1H), 7.18 (dd, *J* = 9.5 Hz, *J* = 2.0 Hz, 1H), 7.05 (s, 1H), 5.54 (s, 2H), 4.73 (t, *J* = 6.0 Hz, 2H), 4.12-4.08 (m, 1H), 3.90 (s, 3H), 2.69 (t, *J* = 6.0 Hz, 2H), 2.40-2.28 (m, 2H) 2.12-2.06 (m, 2H), 2.05-2.00 (m, 1H), 1.89-1.82 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1, 164.8, 153.7, 146.5, 142.0, 139.8, 133.0, 131.4, 120.3, 119.4, 115.6, 114.2, 108.8, 108.5, 104.0, 96.7, 92.5, 78.8, 53.4, 44.7, 37.2, 36.1, 27.5, 21.5, 17.6; HRMS (FAB+) Calcd for $[M]^+$, 632.1373, found for 632.1384.



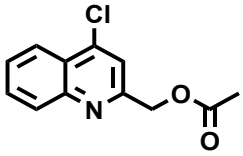
4-chloroquinoline-2-carbaldehyde (compound 18)

Under a nitrogen atmosphere, selenium dioxide (1370 mg, 12.4 mmol) was added to a solution of 4-chloro-2-methylquinoline (2000 mg, 11.3 mmol) in dioxane (120 mL). The reaction was stirred for 4 h at 85 °C, then cooled, diluted with methanol, and vacuum filtered. The filtrate was collected and concentrated, leaving a yellow solid, which was purified by column chromatography (2:8 EtOAc/hexane) to provide compound 18 (1990 mg, 10.4 mmol) as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.2 (s, 1H), 8.31 (ddd, *J* = 8.5 Hz, *J* = 1.5 Hz, *J* = 0.5 Hz, 1H), 8.28 (ddd, *J* = 8.5 Hz, *J* = 1.5 Hz, *J* = 0.5 Hz, 1H), 8.10 (d, *J* = 9.0 Hz, 1H), 7.89 (ddd, *J* = 8.5 Hz, *J* = 7.0 Hz, *J* = 1.5 Hz, 1H), 7.80 (ddd, *J* = 8.5 Hz, *J* = 7.0 Hz, *J* = 1.5 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.6, 152.3, 148.7, 144.2; HRMS (FAB+) Calcd for $[M]^+$, 192.0216, found for 192.0217.



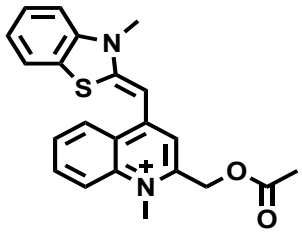
(4-chloroquinolin-2-yl)methanol (compound 19)

$NaBH_4$ was added to a solution of compound 18 (1990 mg, 10.4 mmol) in methanol (300 mL). The reaction mixture was stirred for 15 min, diluted with EtOAc, washed with water followed by brine. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to afford compound 19 (1610 mg, 8.33 mmol) as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.80-7.77 (m, 1H), 7.66-7.62 (m, 1H), 7.43 (s, 1H), 4.90 (s, 2H), 4.21 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 147.6, 143.3, 131.1, 129.0, 127.3, 125.7, 124.2, 118.6, 64.0; HRMS (FAB+) Calcd for $[M+H]^+$,194.0373, found for 194.0371.



(4-chloroquinolin-2-yl)methyl acetate (compound 20)

Compound 19 (400 mg, 2.07 mmol) was dissolved in pyridine (5 mL). To the solution, acetic anhydride (196 µL, 2.07 mmol) was added. After being stirred at room temperature for 4 h, the reaction mixture was concentrated in vacuo, followed by flash chromatography to afford compound 20 as a colorless solid (220 mg, 0.936 mmol): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.5 Hz, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.80-7.77 (m, 1H), 7.67-7.65 (m, 1H), 7.58 (s, 1H), 5.36 (s, 2H, e), 2.22 (s, 3H, g); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.5, 155.3, 147.4, 142.4, 129.7, 128.5, 126.6, 124.7, 123.0, 118.5, 65.9, 19.9; HRMS (FAB+) Calcd for $[M+H]^+$, 236.0478, found for 236.0476.



(*Z*)-2-(acetoxymethyl)-1-methyl-4-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)quinolin-1-ium (compound 21)

To a solution of compound 20 (200 mg, 0.111 mmol) in DCM (10 mL) was added dropwise methyl trifluoromethanesulfonate (172 µL, 1.57 mmol). After being stirred at room temperature for 4 h, the reaction mixture was concentrated in vacuo, which was used in the next step without further purification. To a solution of the above quinolinium in ethanol (2.5 mL) was added 2,3-dimethylbenzo[*d*]thiazol-3-ium (117 mg, 0.710 mmol) and *N*,*N*-diisopropylethylamine (124 µL, 0.710 mmol). After being stirred at room temperature for 3 h in the dark, the solvent was removed under reduced pressure. The residue was used in the next step without further purification. To a solution of crude in DCM (4.00 mL) was added TFA (1.00 mL). After stirring for 2 h, a solvent was removed under reduced pressure. The residue was purified by reversed-phase HPLC under the following conditions: A/B = 75/25 (0 min), 30/70 (30 min), (solvent A: 0.1% HCOOH in H_2O; solvent B: 0.1% HCOOH in CH_3CN). After lyophilization, an orange powder of compound 21 (11.2 mg, 0.0332 mmol) was obtained: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (d, *J* = 8.0 Hz, 1H), 8.17 (d, *J* = 8.5 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 8.03-7.99 (m, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.75 (m, 1H), 7.65-7.61 (m, 1H),7.46-7.43 (m, 1H), 7.43 (s, 1H, e), 6.96 (s, 1H), 5.59 (s, 2H), 4.04 (s, 6H), 2.31 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 160.8, 150.3, 149.0, 141.0, 139.7, 134.0, 128.8, 127.2, 125.9, 125.2, 124.4, 123.8, 123.5, 118.7, 113.7, 108.2, 89.0, 63.0, 37.1, 34.5, 21.2; HRMS (FAB+) Calcd for $[M]^+$, 377.1318, found for 377.1333.

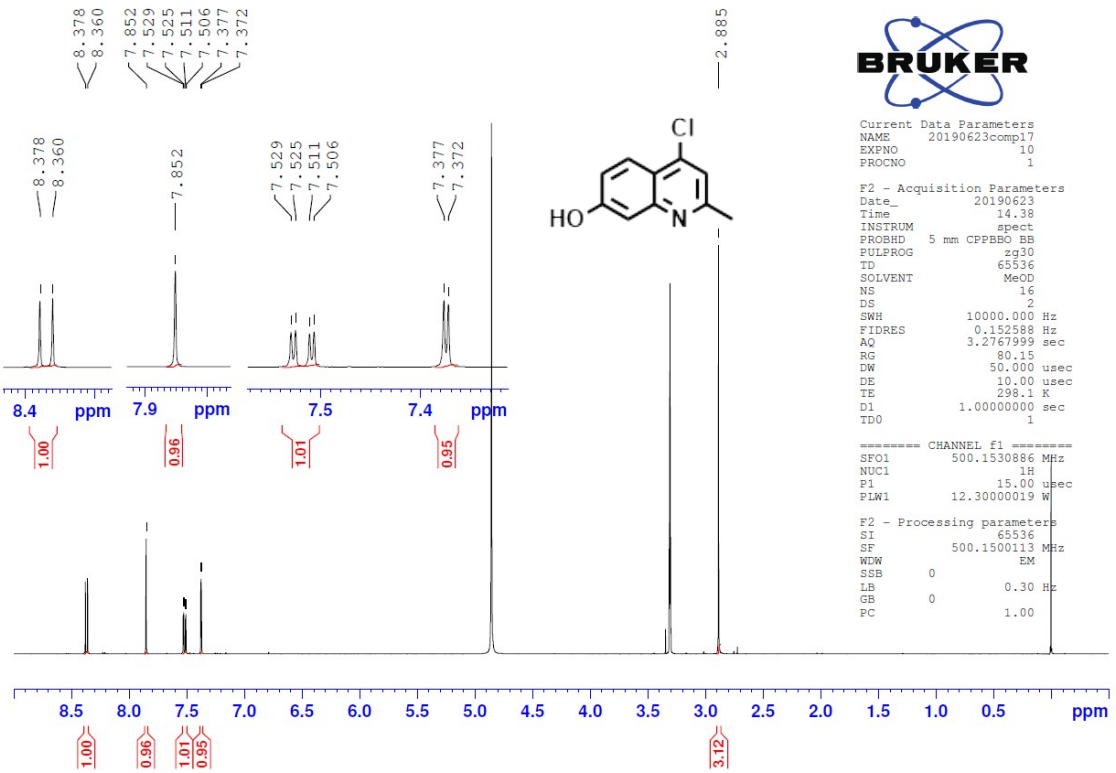


Figure S16. ^{1}H NMR spectrum of compound 4 in MeOD

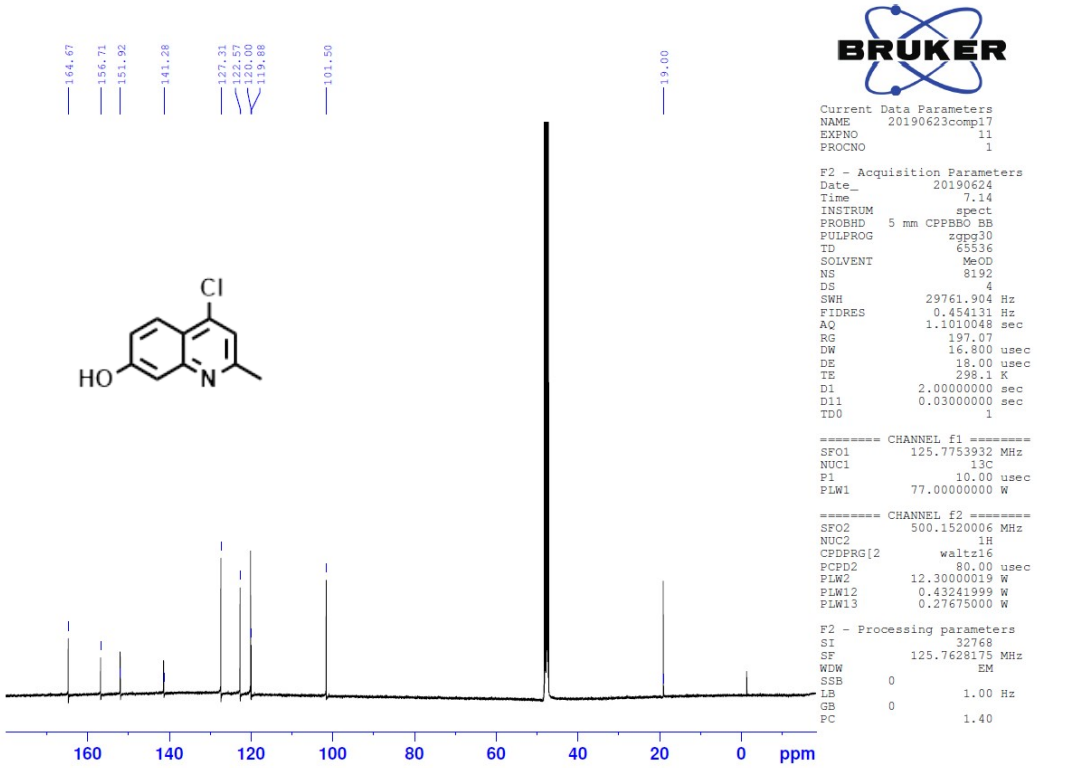


Figure S17. ^{13}C NMR spectrum of compound 4 in MeOD.

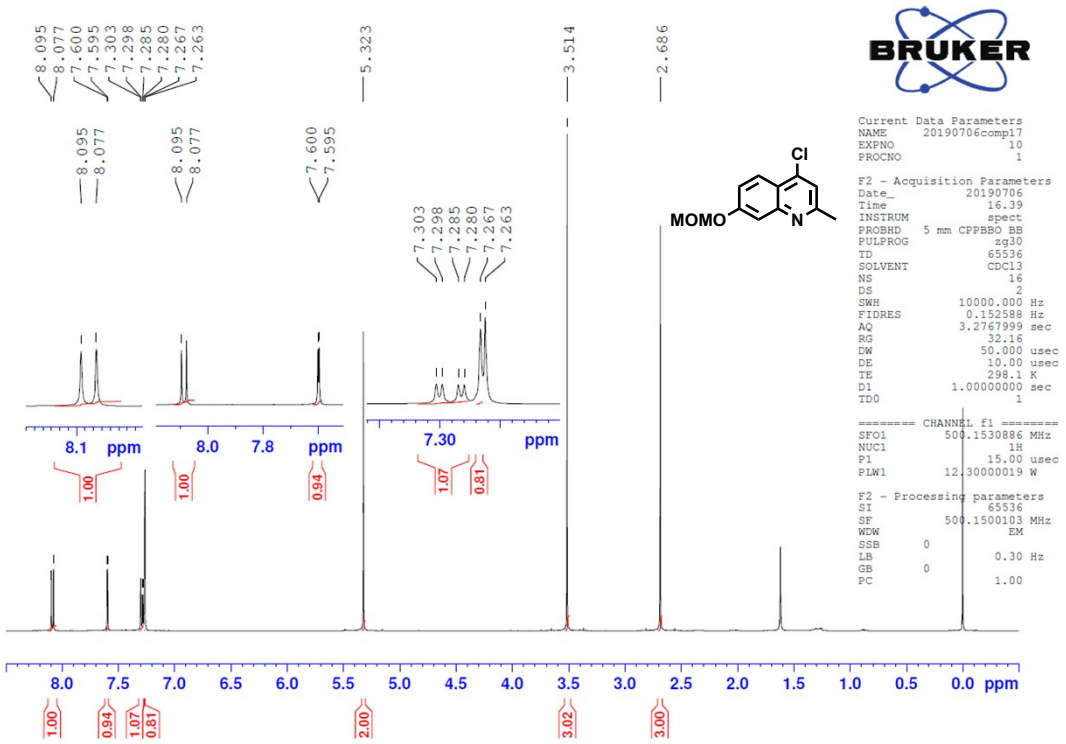


Figure S18. ^{1}H NMR spectrum of compound 5 in $CDCl_3$

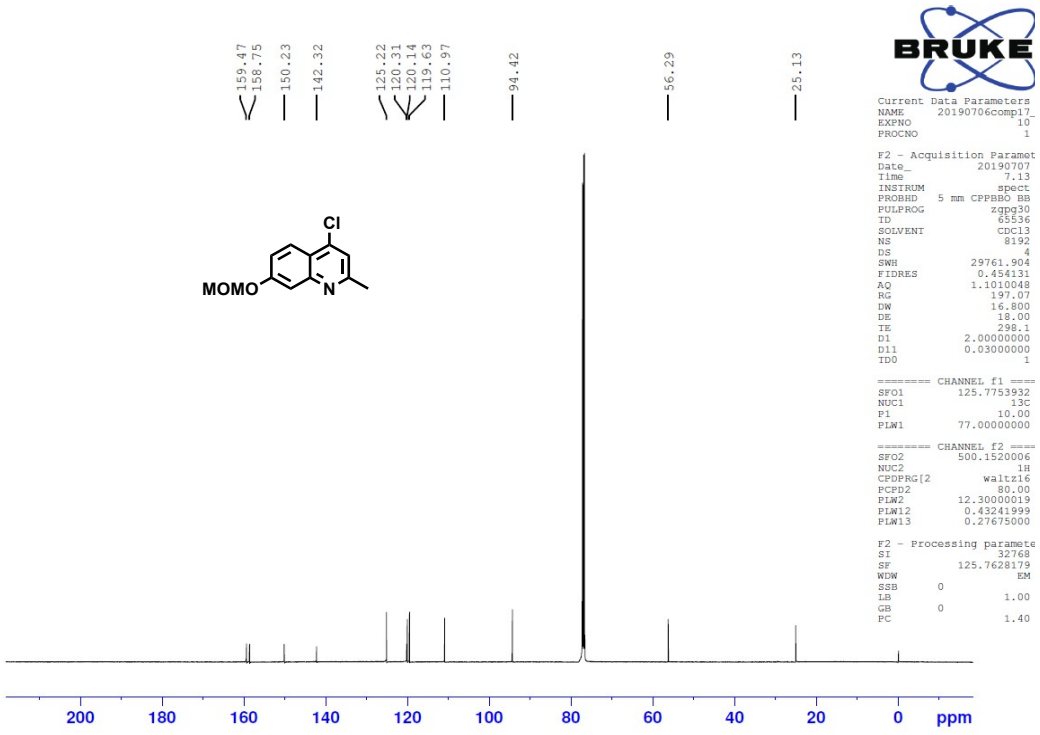


Figure S19. ^{13}C NMR spectrum of compound 5 in $CDCl_3$.

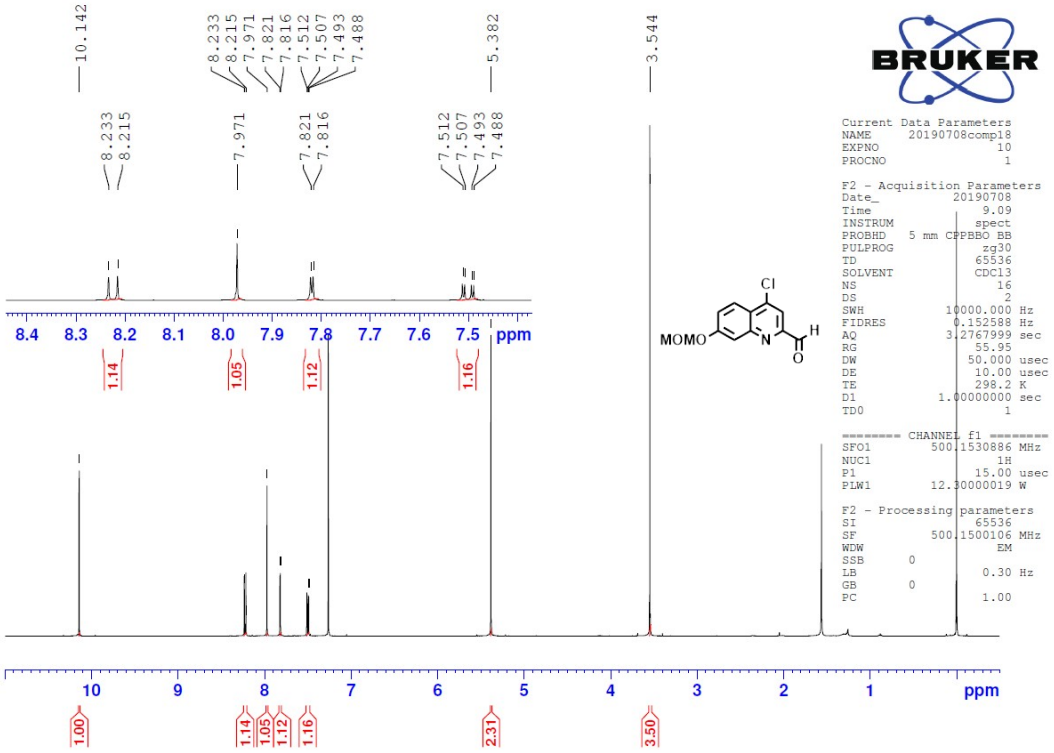


Figure S20. ^{1}H NMR spectrum of compound 6 in $CDCl_3$.

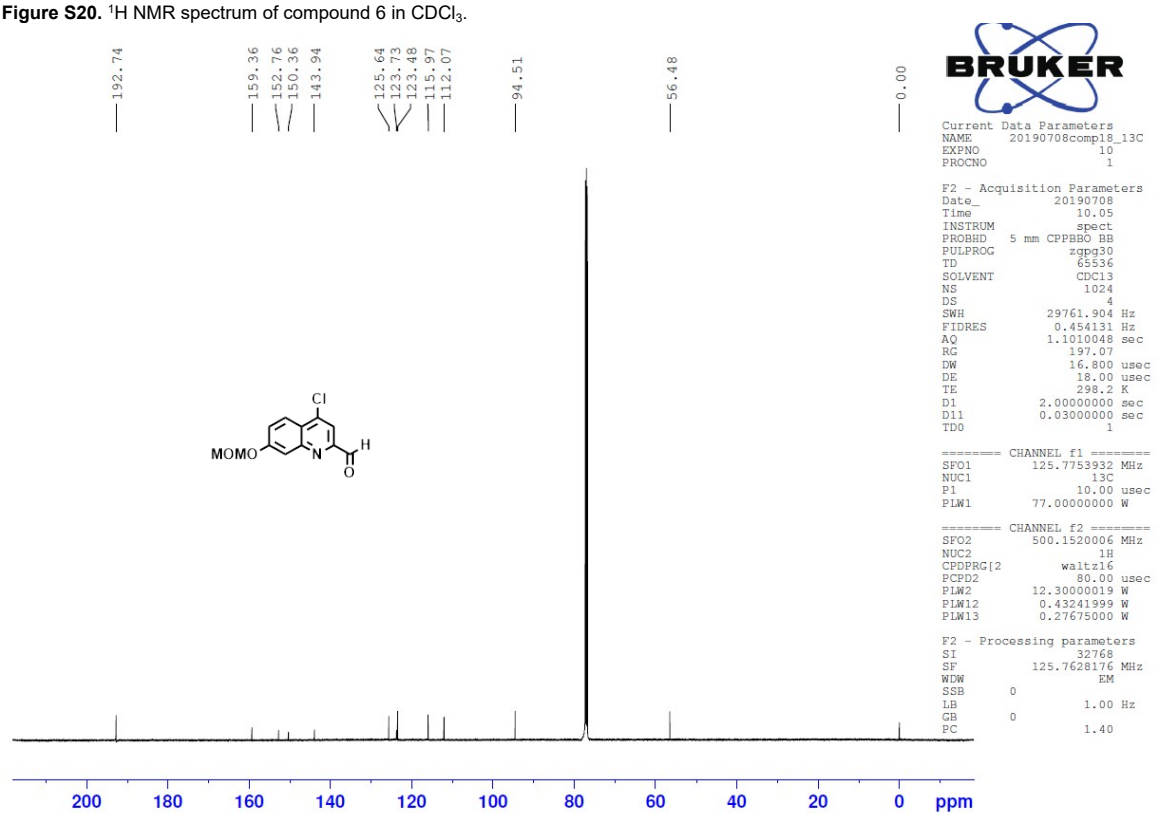


Figure S21. ^{13}C NMR spectrum of compound 6 in $CDCl_3$.

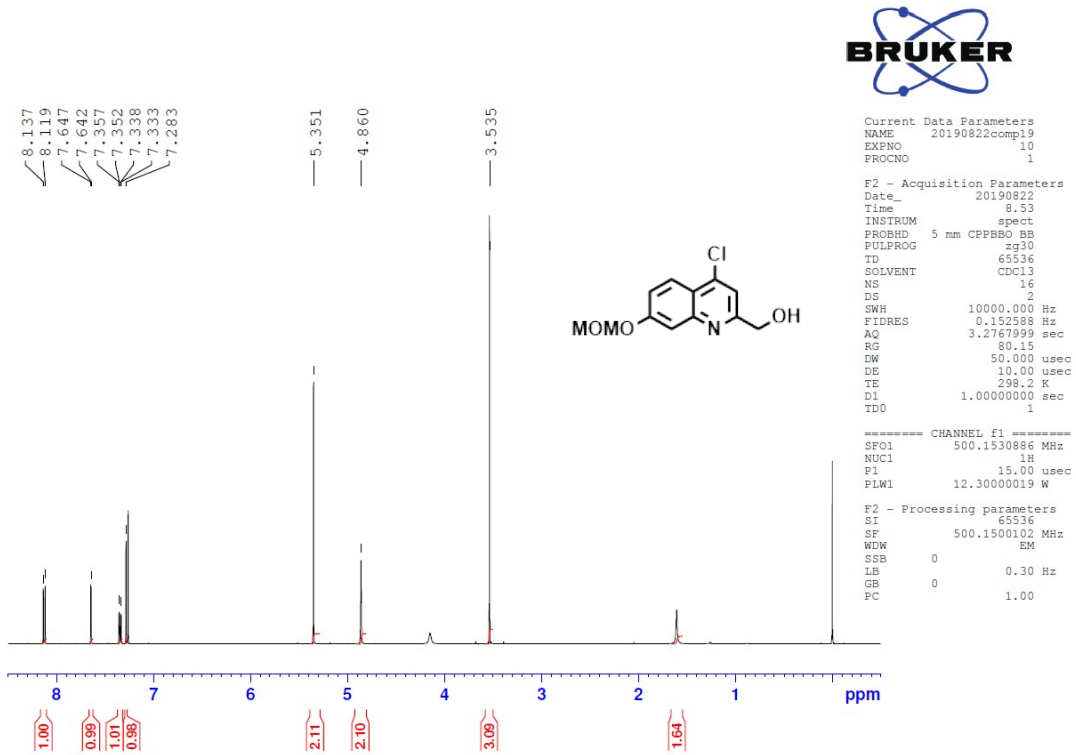


Figure S22. ^{1}H NMR spectrum of compound 7 in $CDCl_3$.

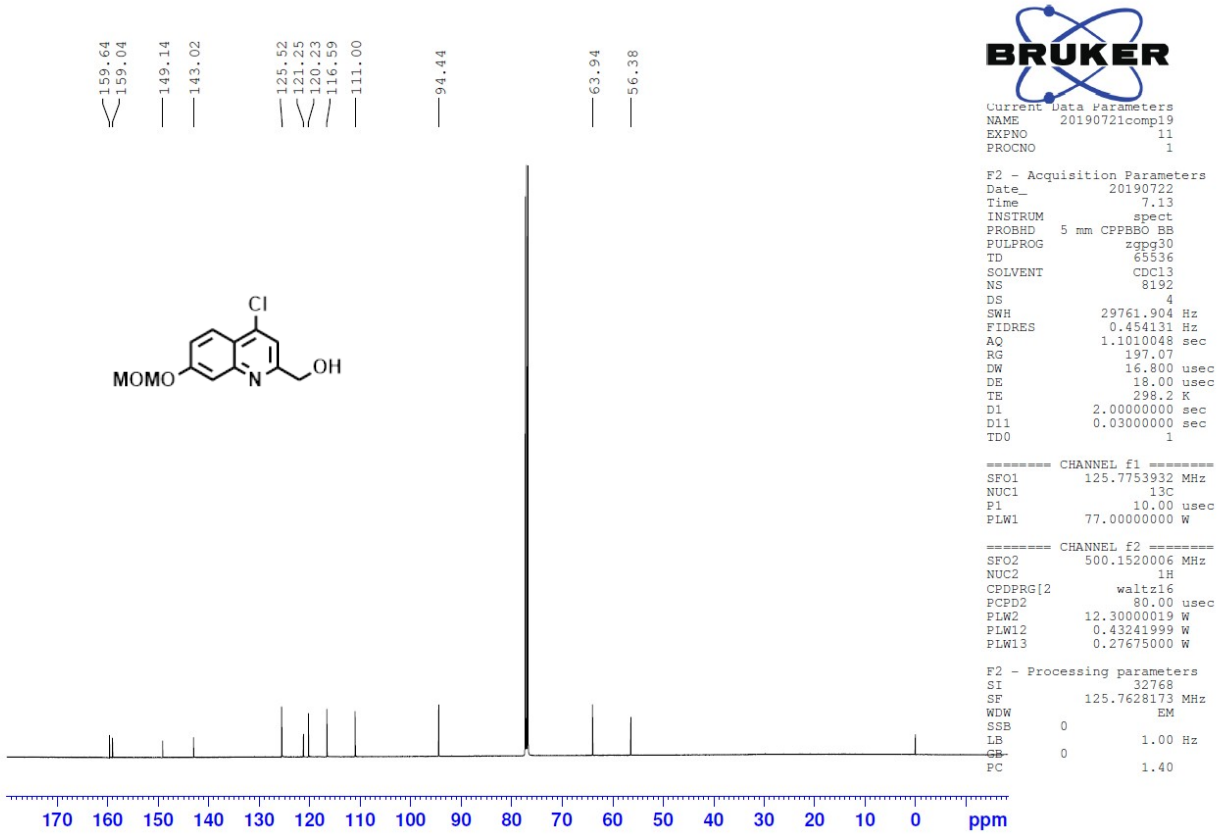


Figure S23. ^{13}C NMR spectrum of compound 7 in $CDCl_3$.

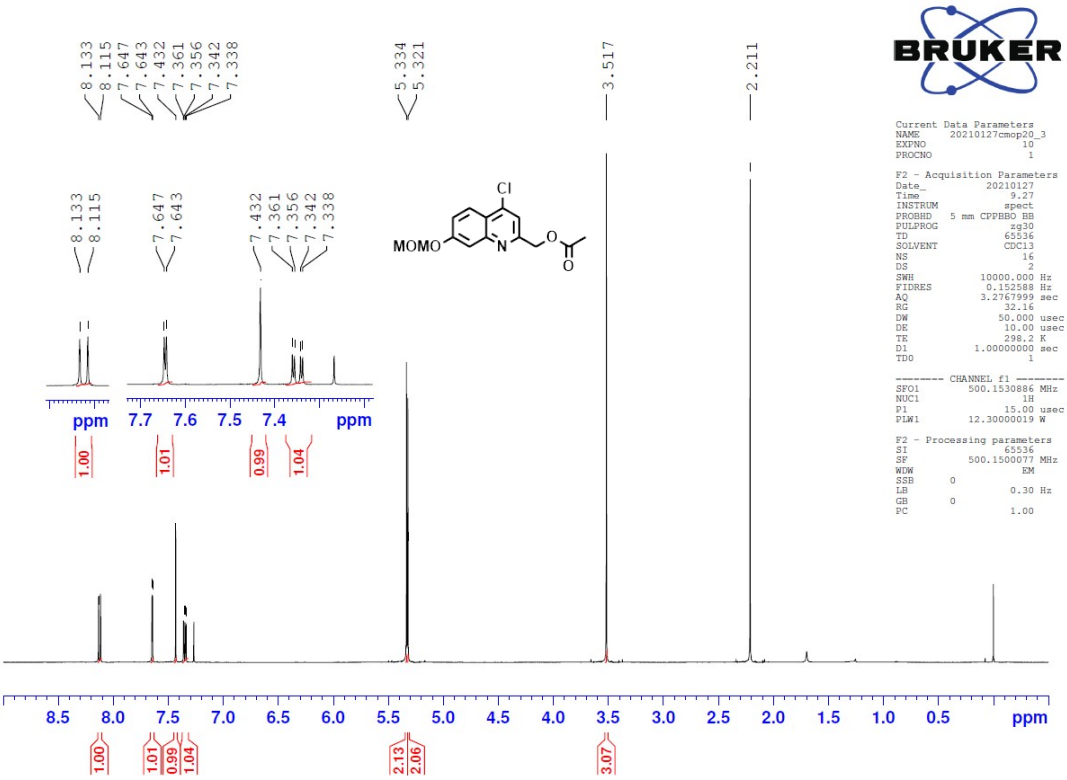


Figure S24. ^{1}H NMR spectrum of compound 8 in $CDCl_3$.

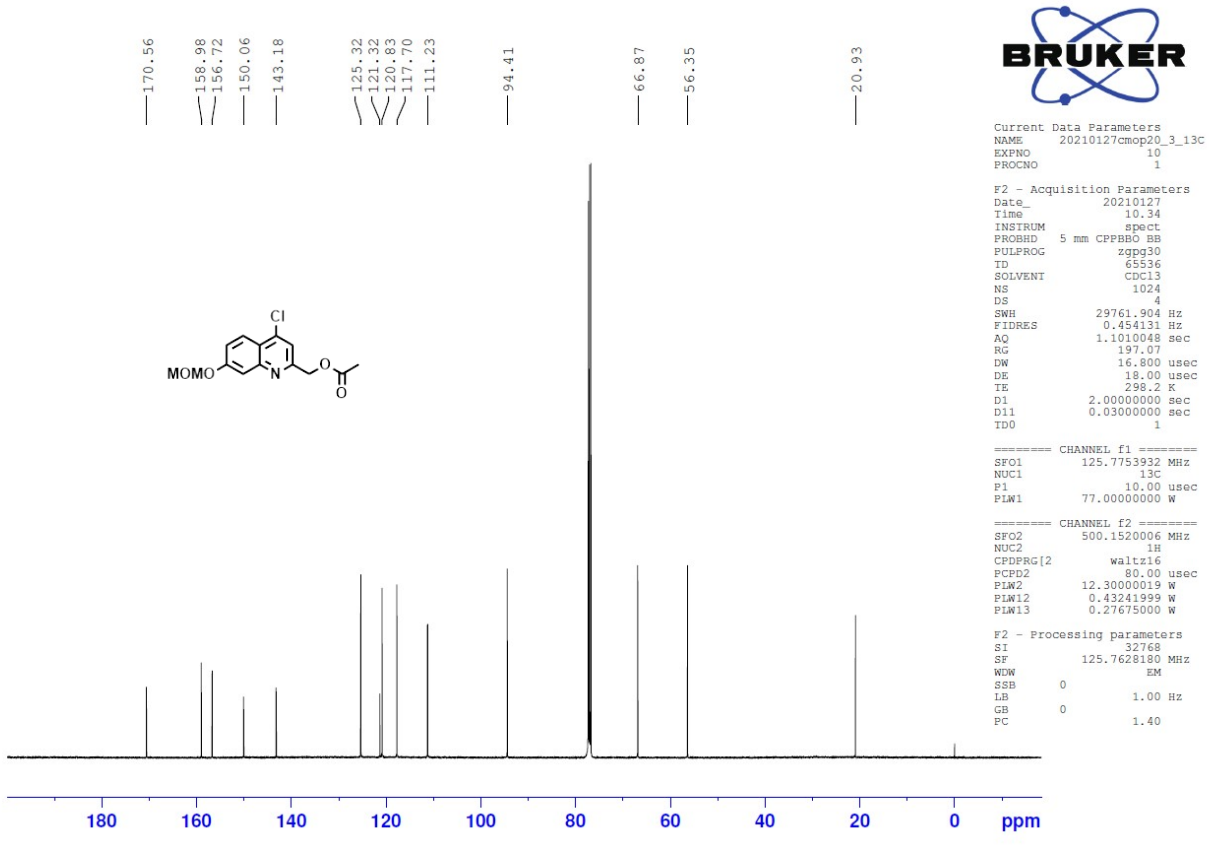


Figure S25. ^{13}C NMR spectrum of compound 8 in $CDCl_3$.

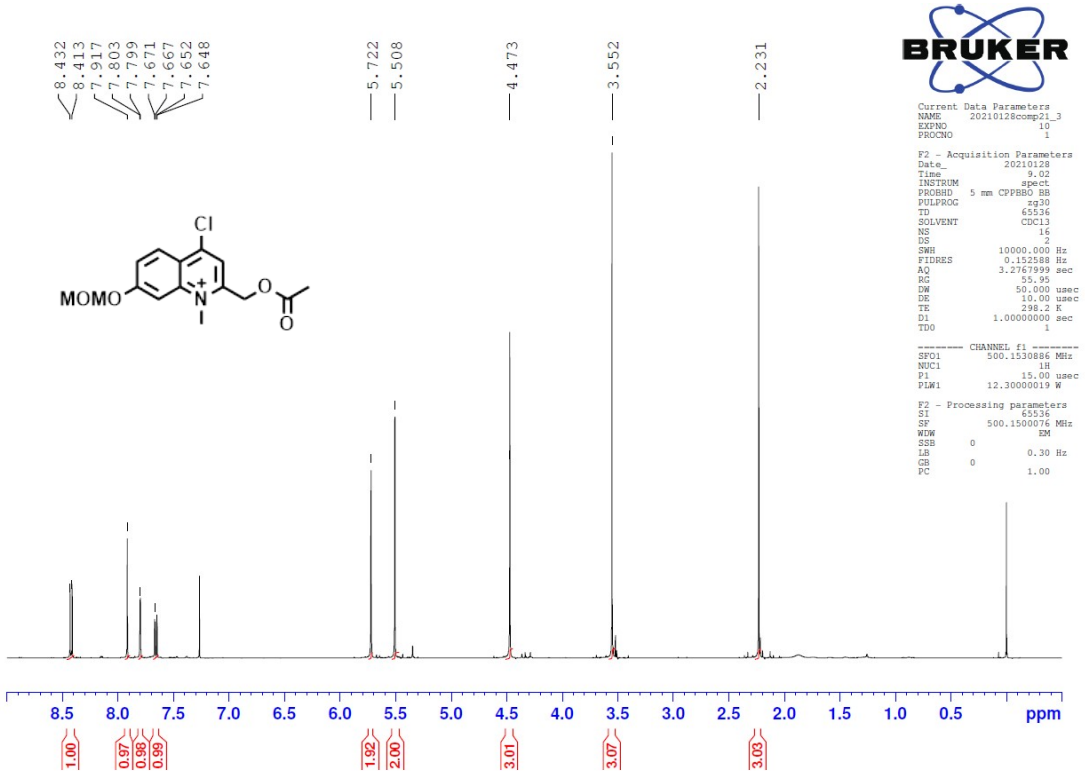


Figure S26. ^{1}H NMR spectrum of compound 9 in $CDCl_3$.

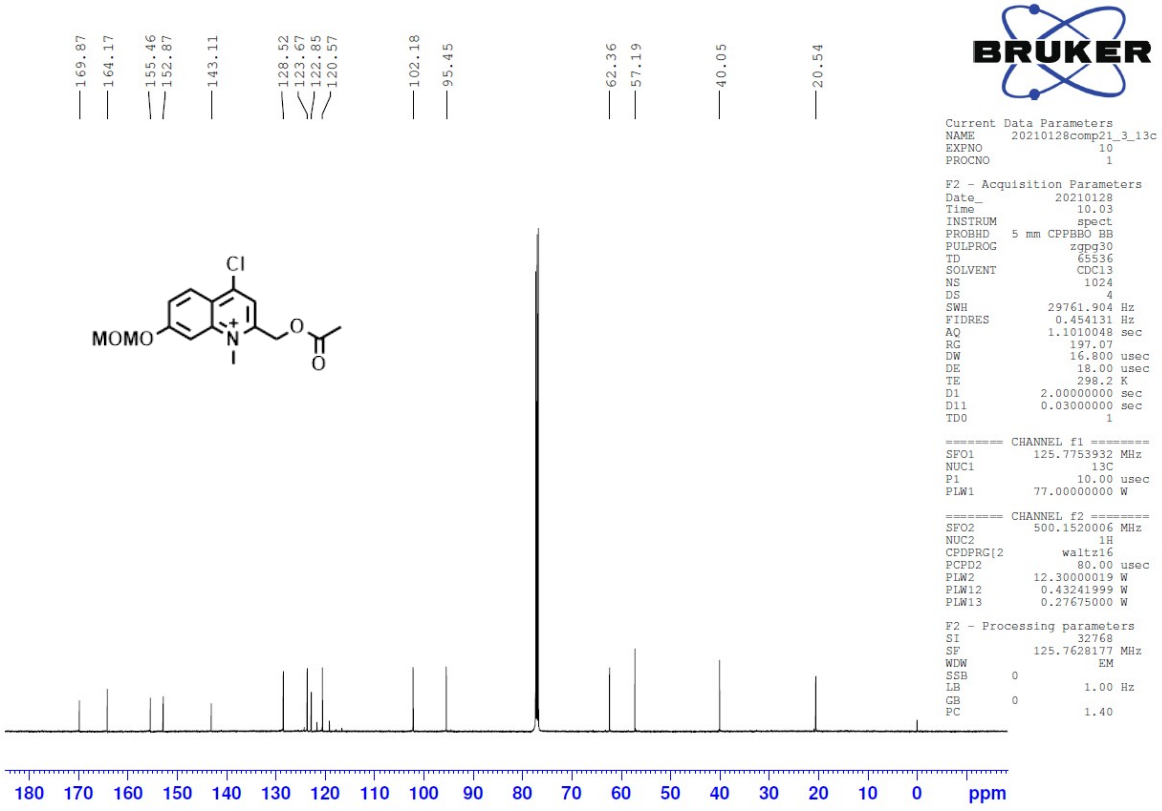


Figure S27. ^{13}C NMR spectrum of compound 9 in $CDCl_3$.

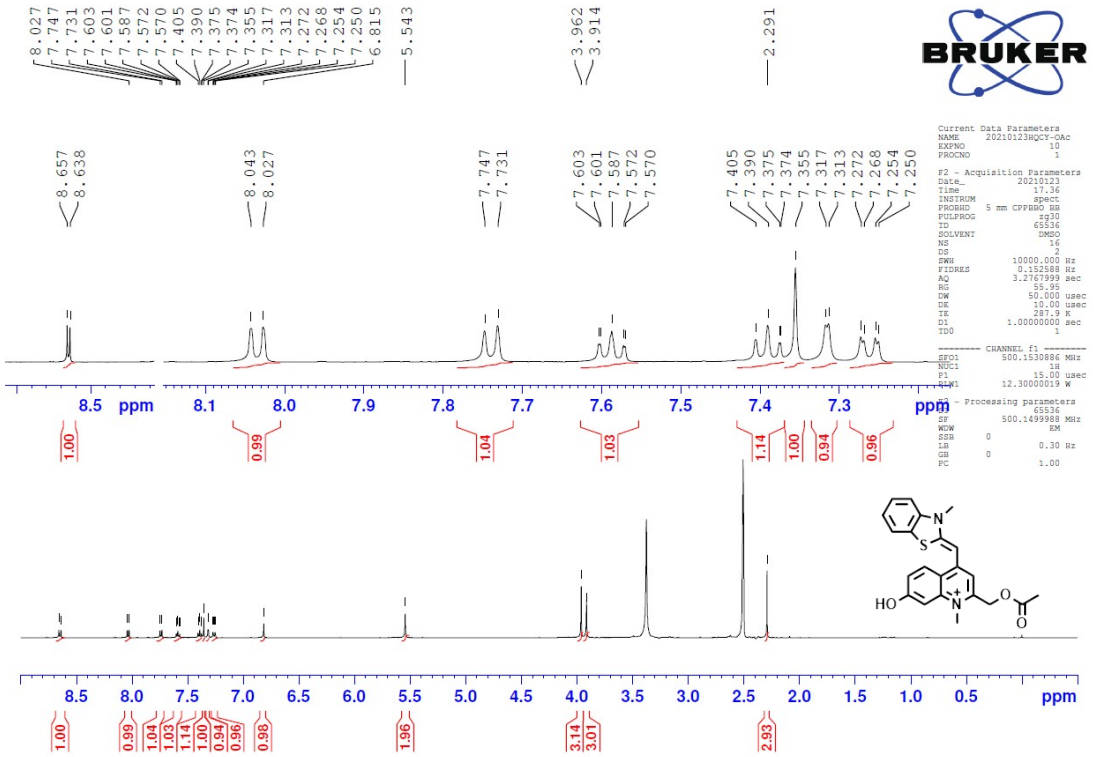


Figure S28. ^{1}H NMR spectrum of compound 11 in DMSO-d_6.

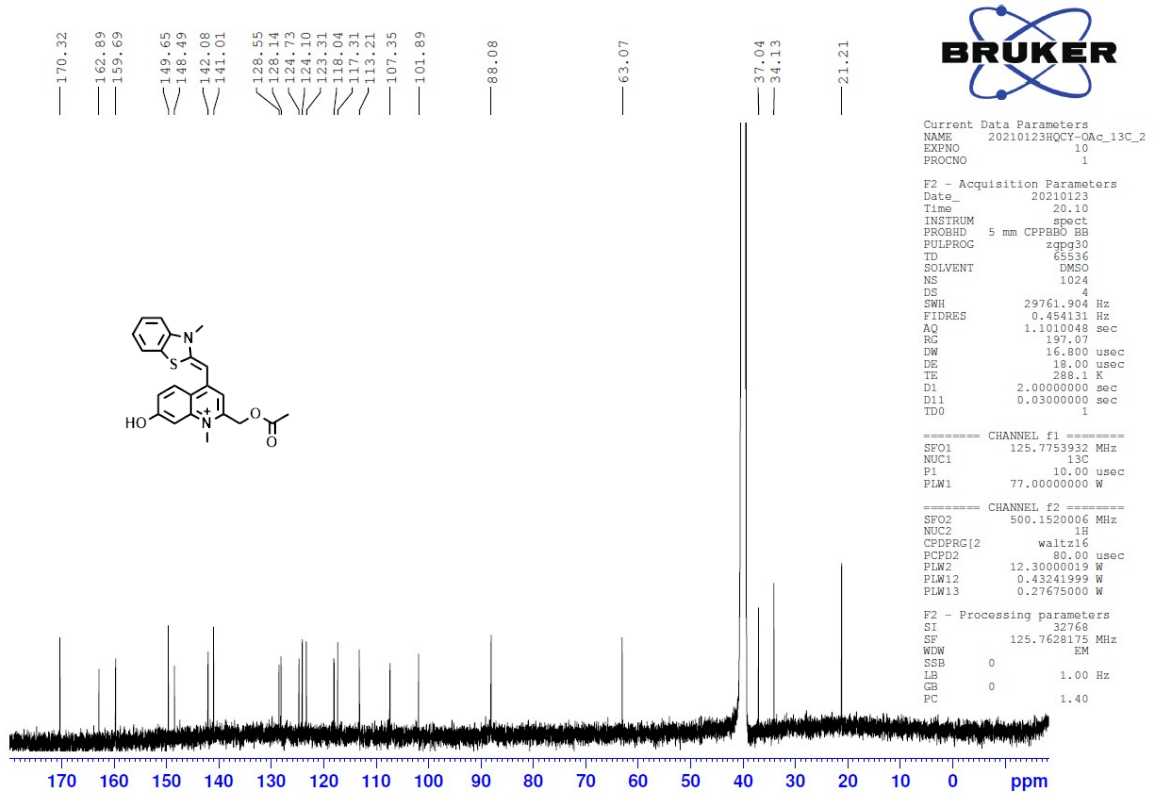


Figure S29. ^{13}C NMR spectrum of compound 11 in DMSO-d_6.

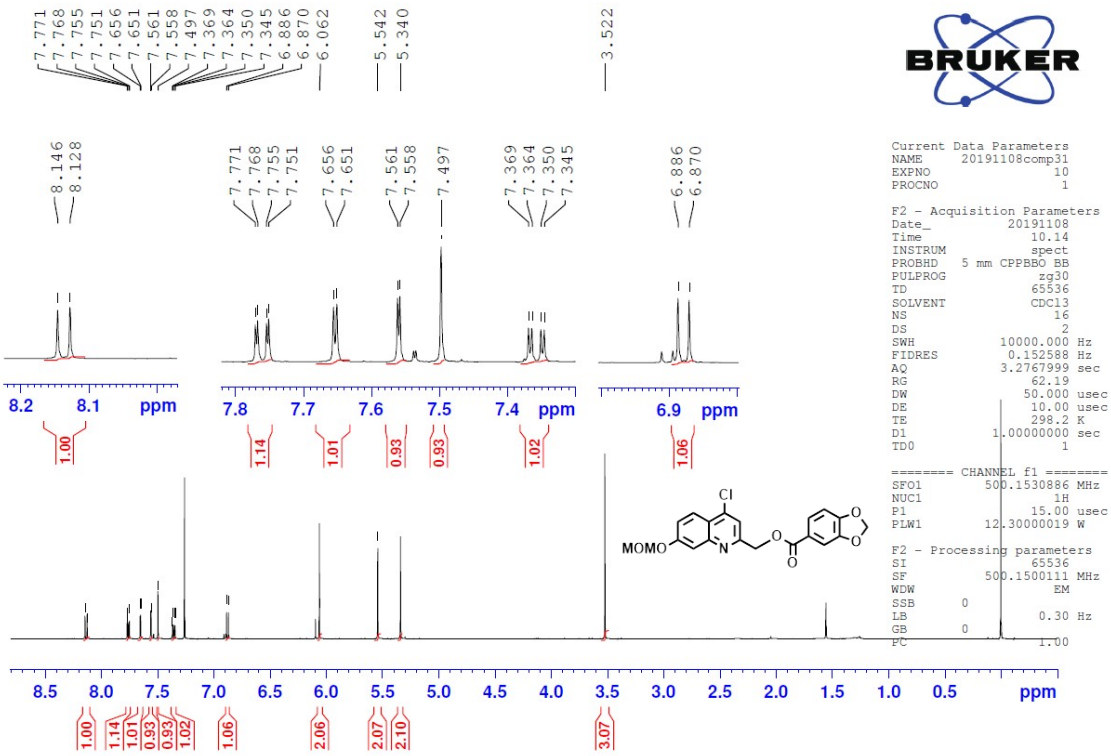



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Figure S30. ^{1}H NMR spectrum of compound 12 in $CDCl_3$.

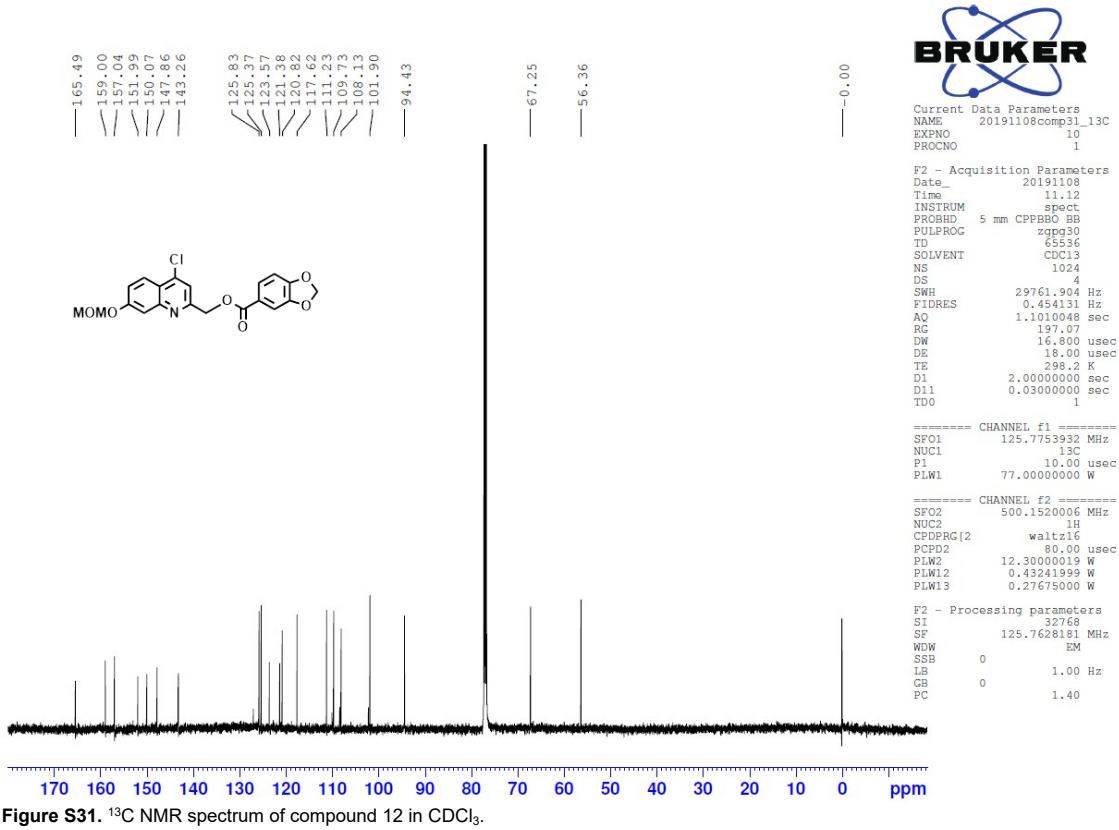


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Figure S31. ^{13}C NMR spectrum of compound 12 in $CDCl_3$.

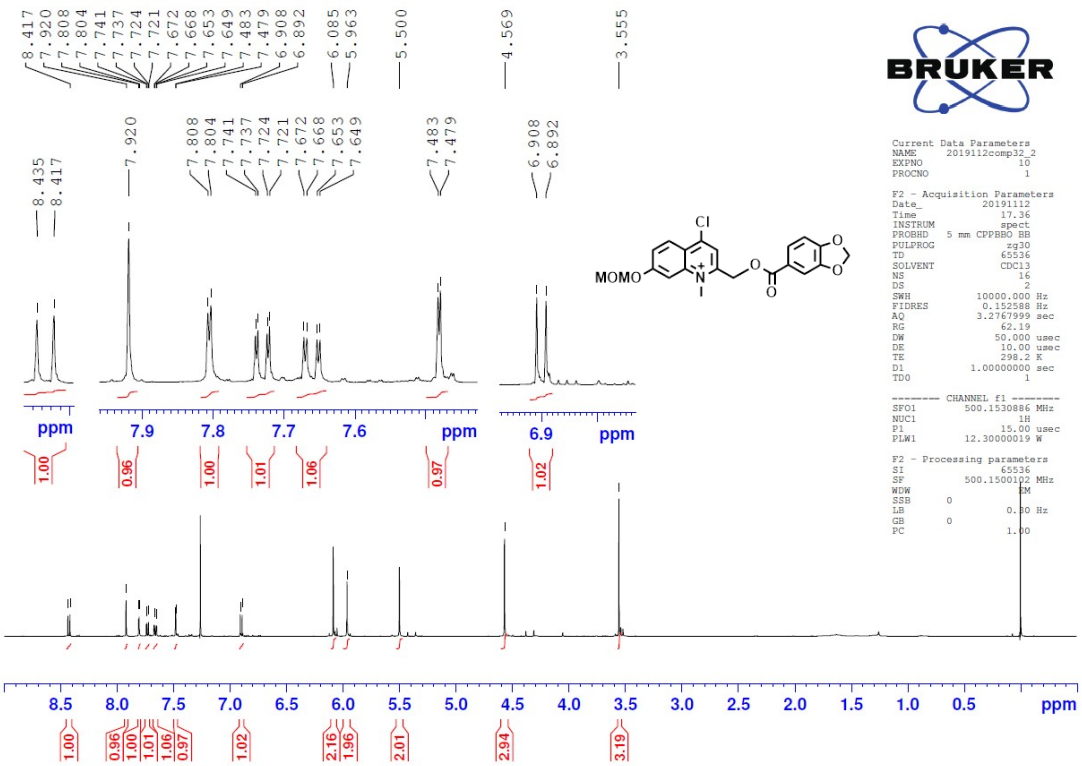


Figure S32. ^{1}H NMR spectrum of compound 13 in $CDCl_3$.

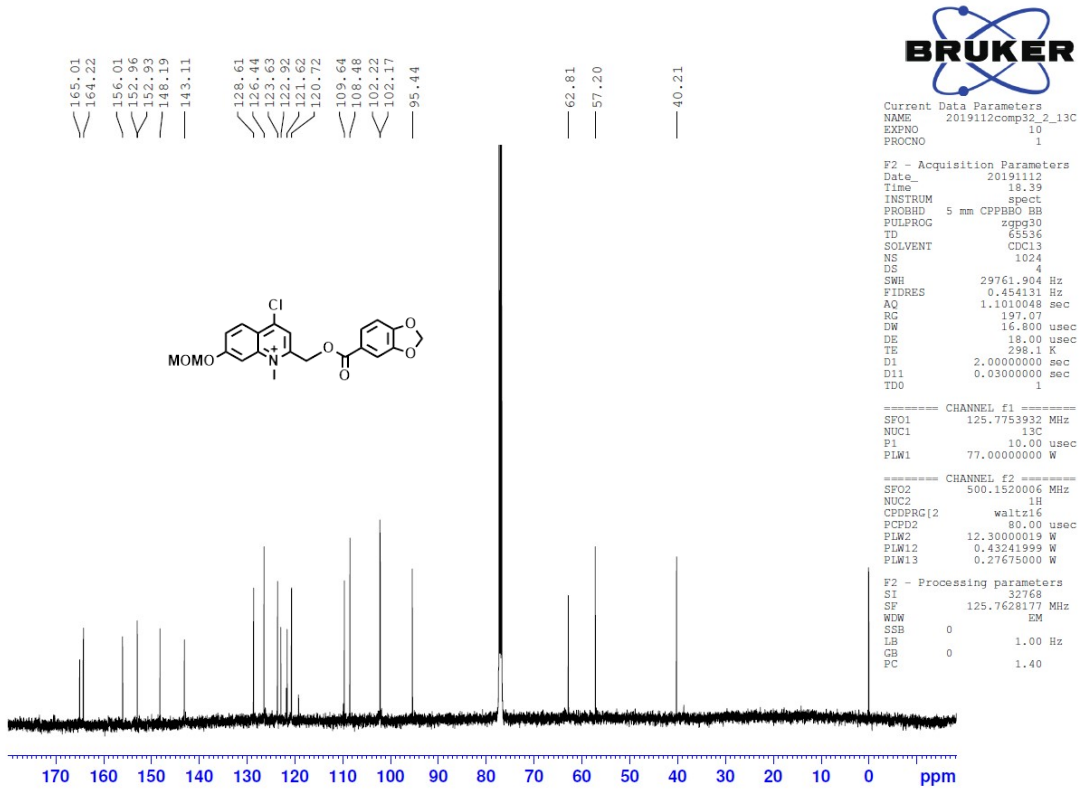


Figure S33. ^{13}C NMR spectrum of compound 13 in $CDCl_3$.

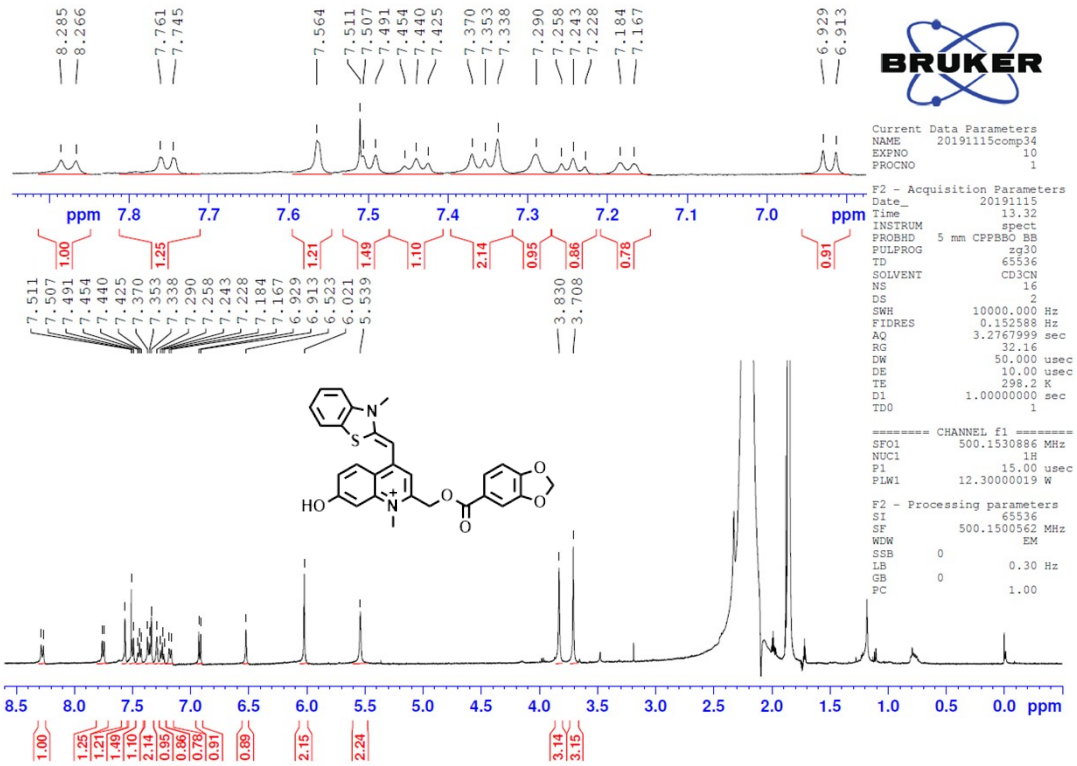


Figure S34. ^{1}H NMR spectrum of compound 14 in CD_3CN.

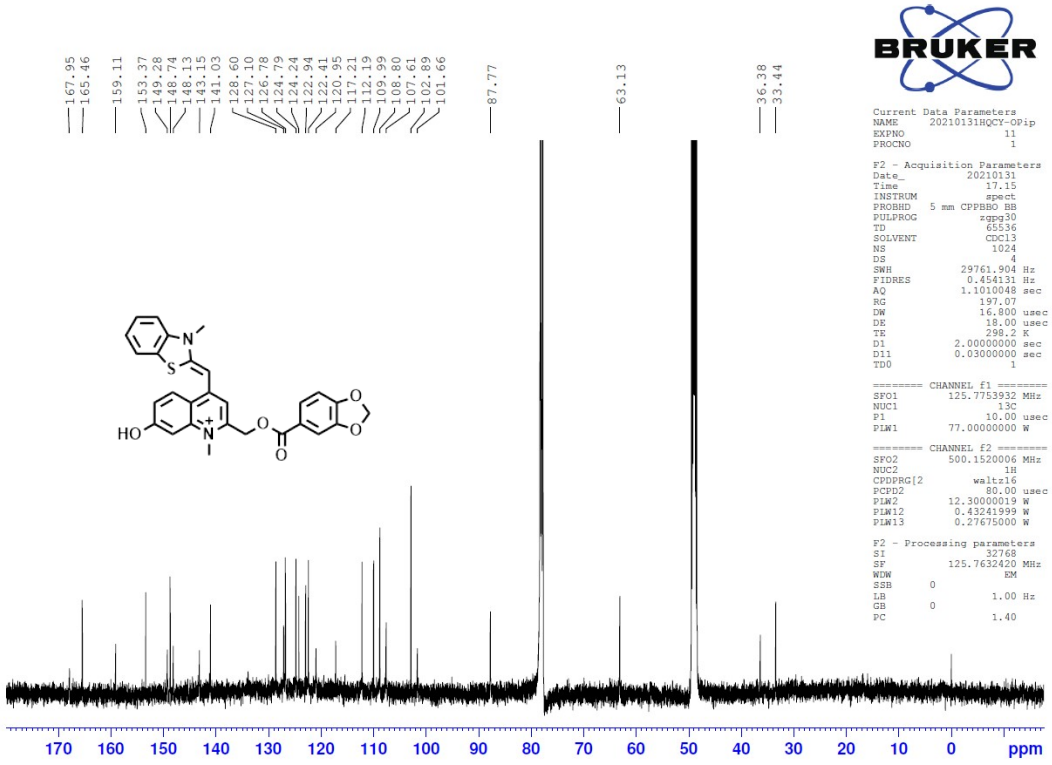


Figure S35. ^{13}C NMR spectrum of compound 14 in $CDCl_3$/MeOD.

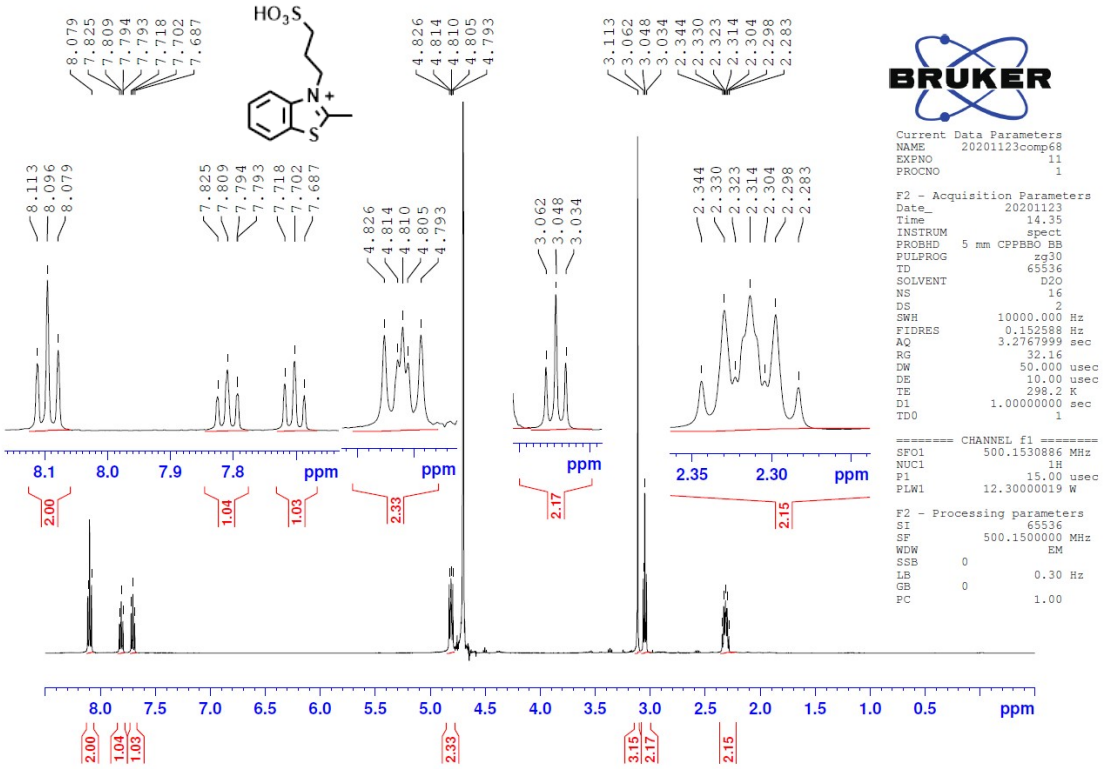


Figure S36. ^{1}H NMR spectrum of compound 15 in D_2O.

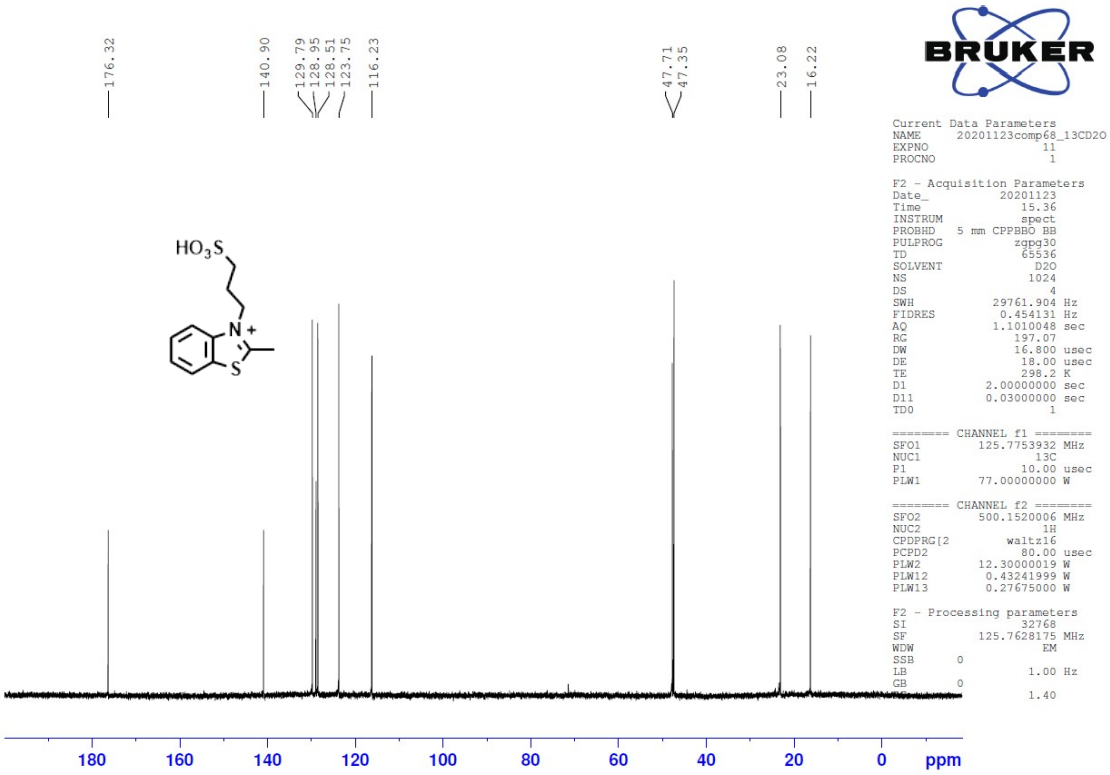


Figure S37. ^{13}C NMR spectrum of compound 15 in D_2O.

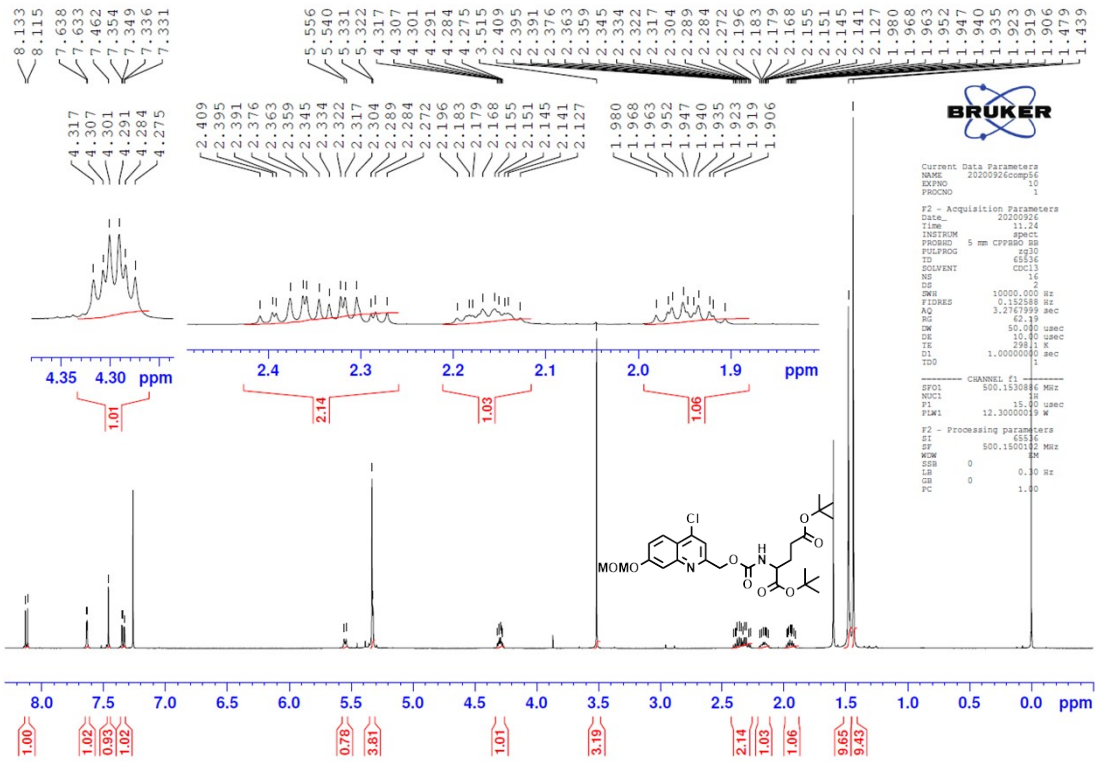


Figure S38. ^{1}H NMR spectrum of compound 16 in $CDCl_3$.

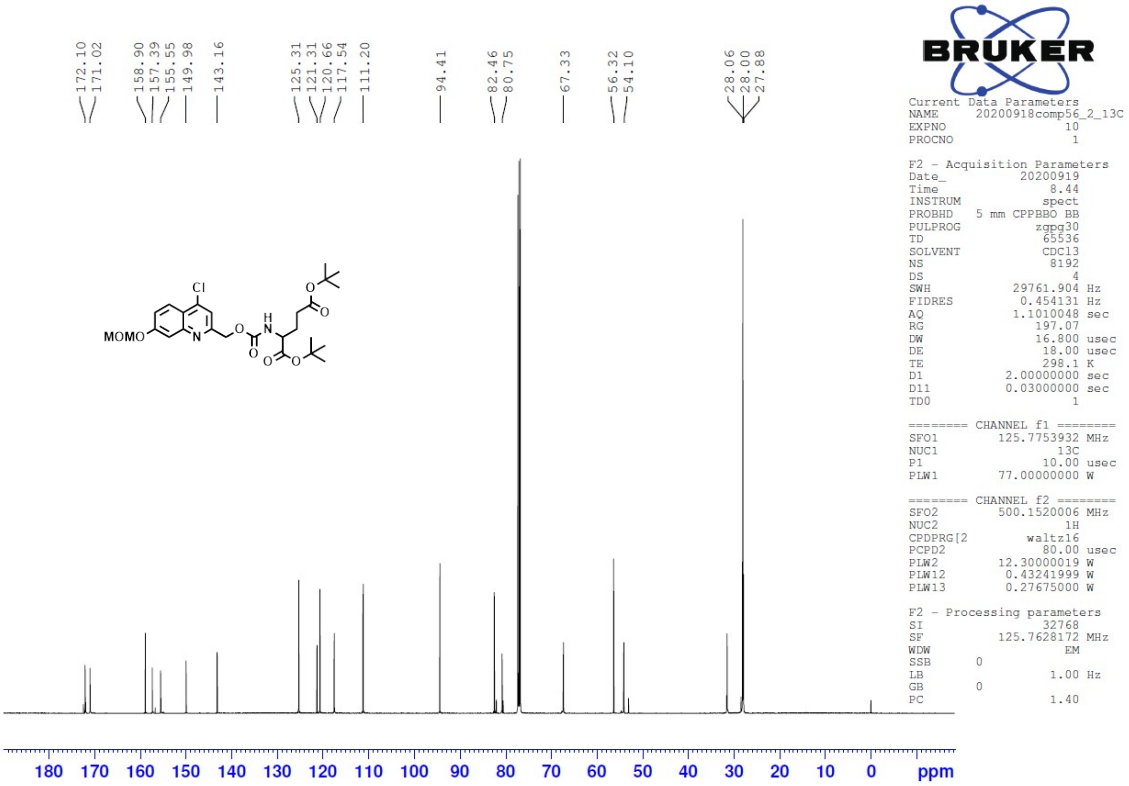


Figure S39. ^{13}C NMR spectrum of compound 16 in $CDCl_3$.

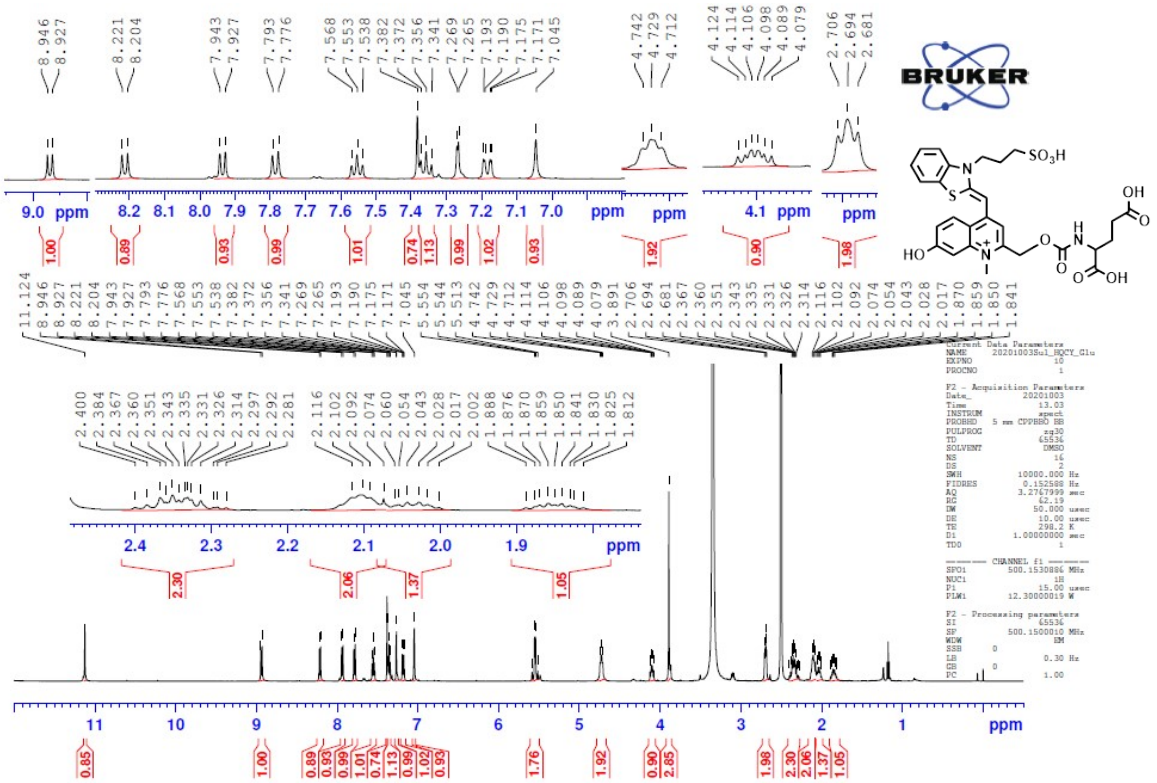


Figure S40. ^{1}H NMR spectrum of compound 17 in DMSO-d_6.

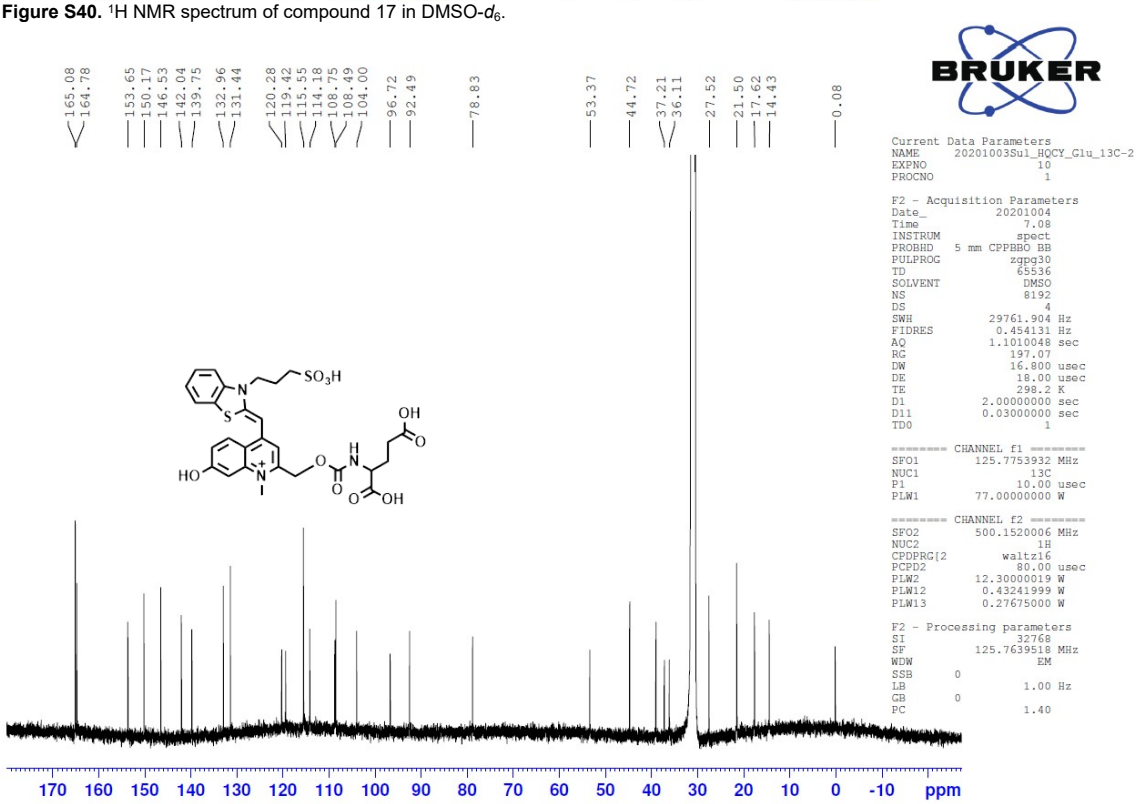
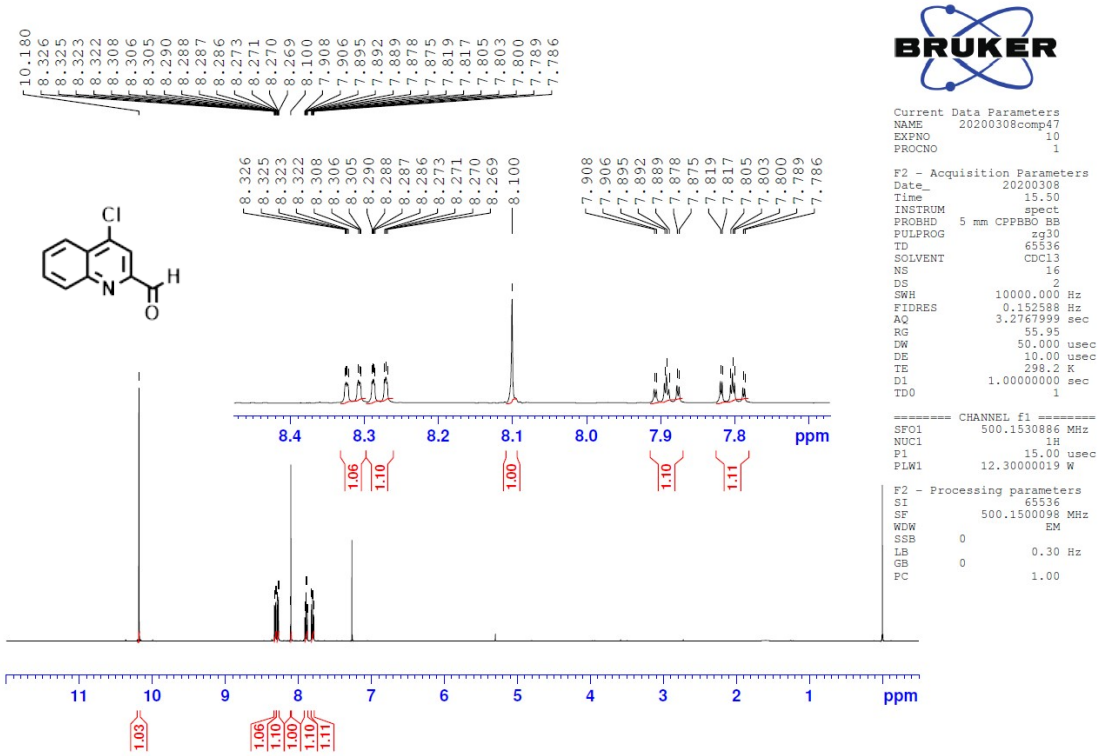


Figure S41. ^{13}C NMR spectrum of compound 17 in DMSO-d_6.

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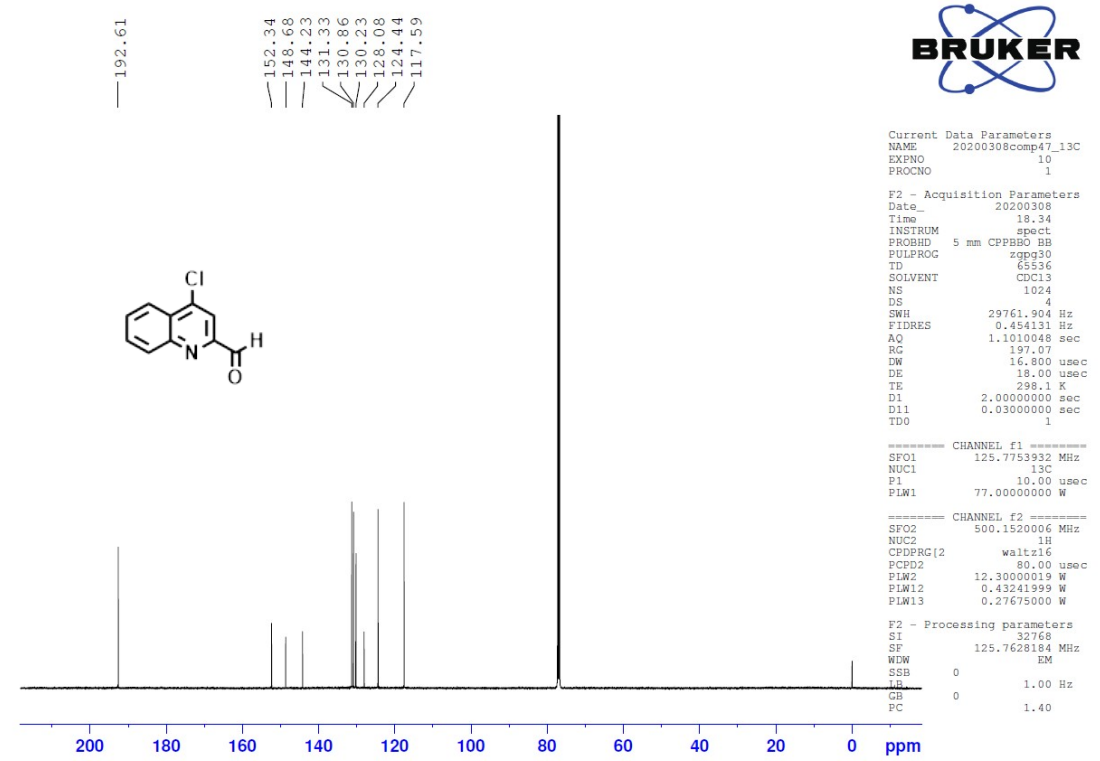
Current Data Parameters
NAME      20200308comp47
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200308
Time               15.50
INSTRUM             spect
PROBHD   5 mm CPPBBO BB
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            10000.000 Hz
FIDRES             0.152588 Hz
AQ                 3.2767999 sec
RG                    55.95
DW                 50.000 usec
DE                 10.00 usec
TE                 298.2 K
D1              1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.1530886 MHz
NUC1                  1H
P1                 15.00 usec
PLW1          12.30000019 W

F2 - Processing parameters
SI                 65536
SF            500.1500098 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

Figure S 42. ^{1}H NMR spectrum of compound 18 in $CDCl_3$.




```
Current Data Parameters
NAME      20200308comp47_13C
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_           20200308
Time               18.34
INSTRUM             spect
PROBHD   5 mm CPPBBO BB
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            29761.904 Hz
FIDRES             0.454131 Hz
AQ                 1.1010048 sec
RG                 197.07
DW                 16.800 usec
DE                 18.00 usec
TE                 298.1 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          125.7753932 MHz
NUC1                  13C
P1                 10.00 usec
PLW1          77.00000000 W

======== CHANNEL f2 ========
SFO2          500.1520006 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          12.30000019 W
PLW12          0.43241999 W
PLW13          0.27675000 W

F2 - Processing parameters
SI                 32768
SF            125.7628184 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Figure S 43. ^{13}C NMR spectrum of compound 18 in $CDCl_3$.

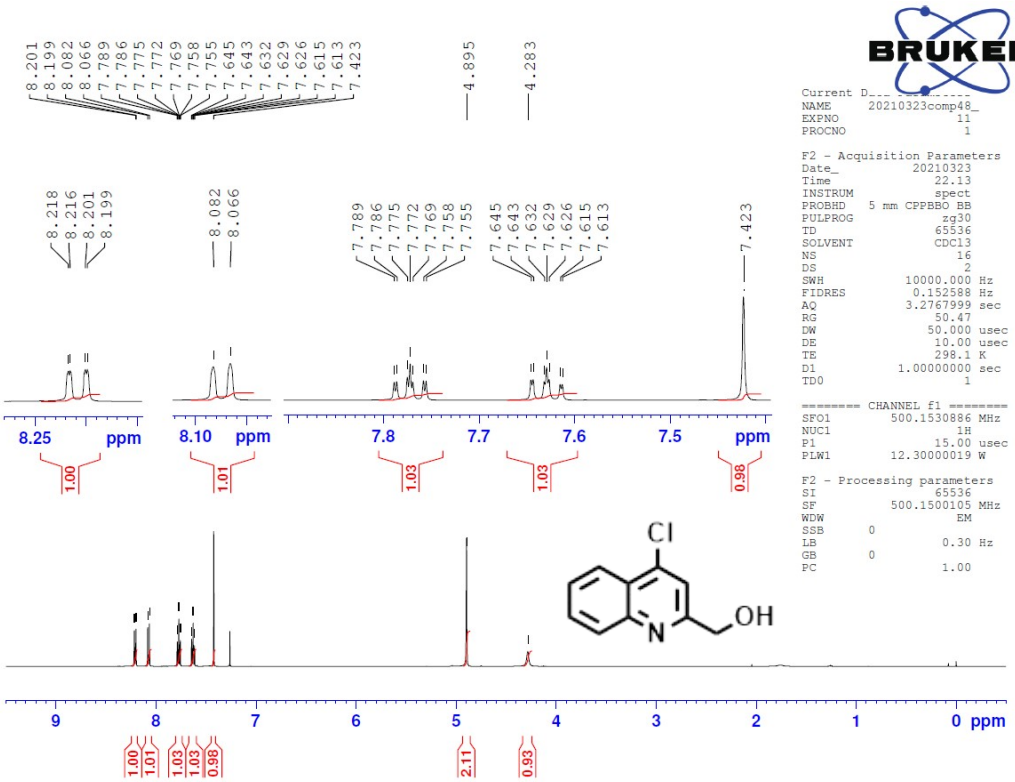


Figure S 44. ^{1}H NMR spectrum of compound 19 in $CDCl_3$.

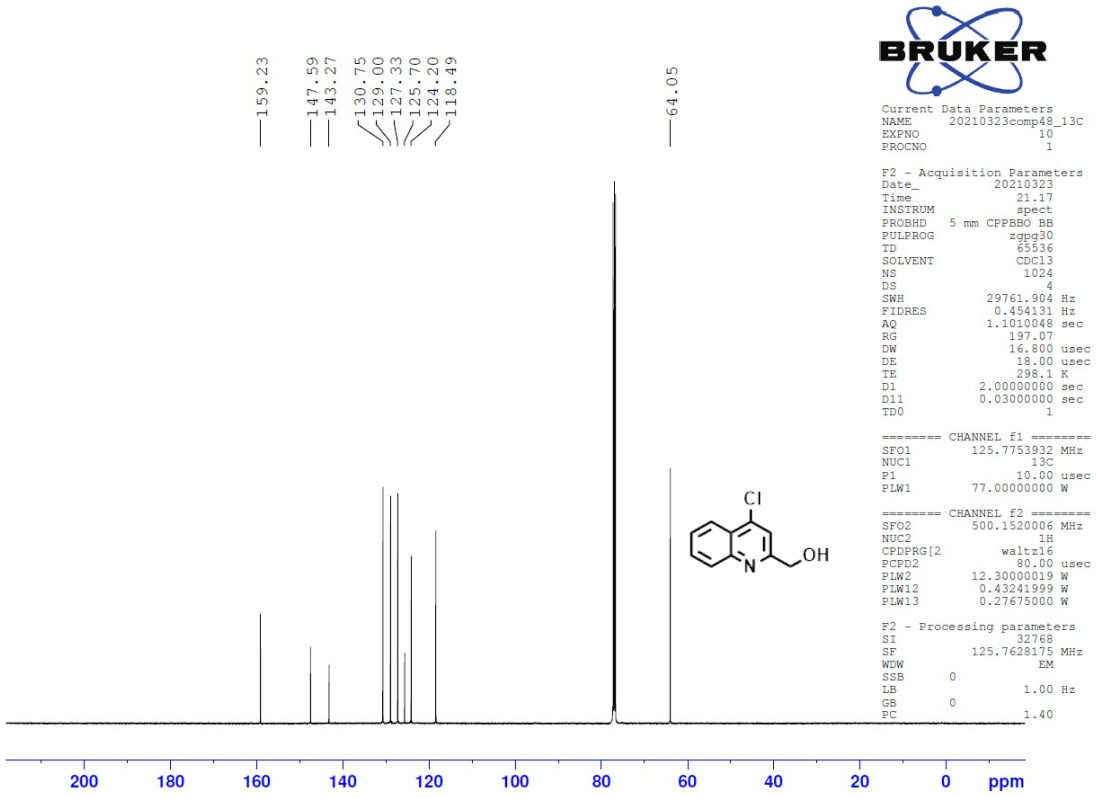


Figure S 45. ^{13}C NMR spectrum of compound 19 in $CDCl_3$.

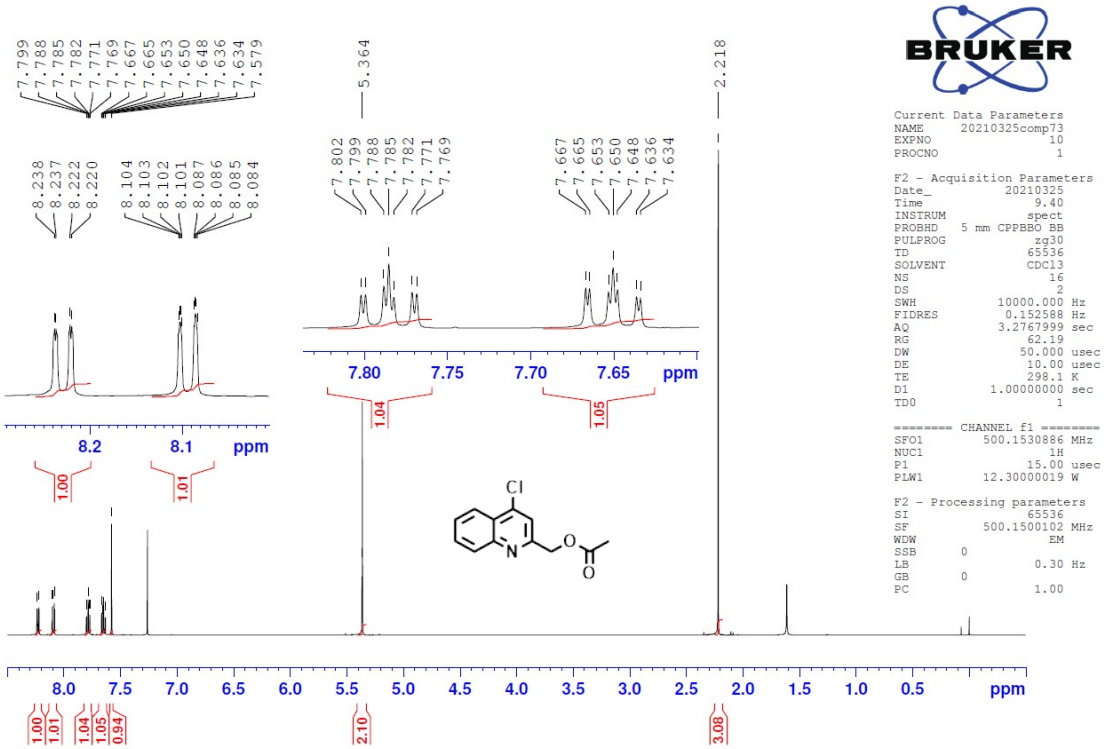


Figure S 46. ^{1}H NMR spectrum of compound 20 in $CDCl_3$.

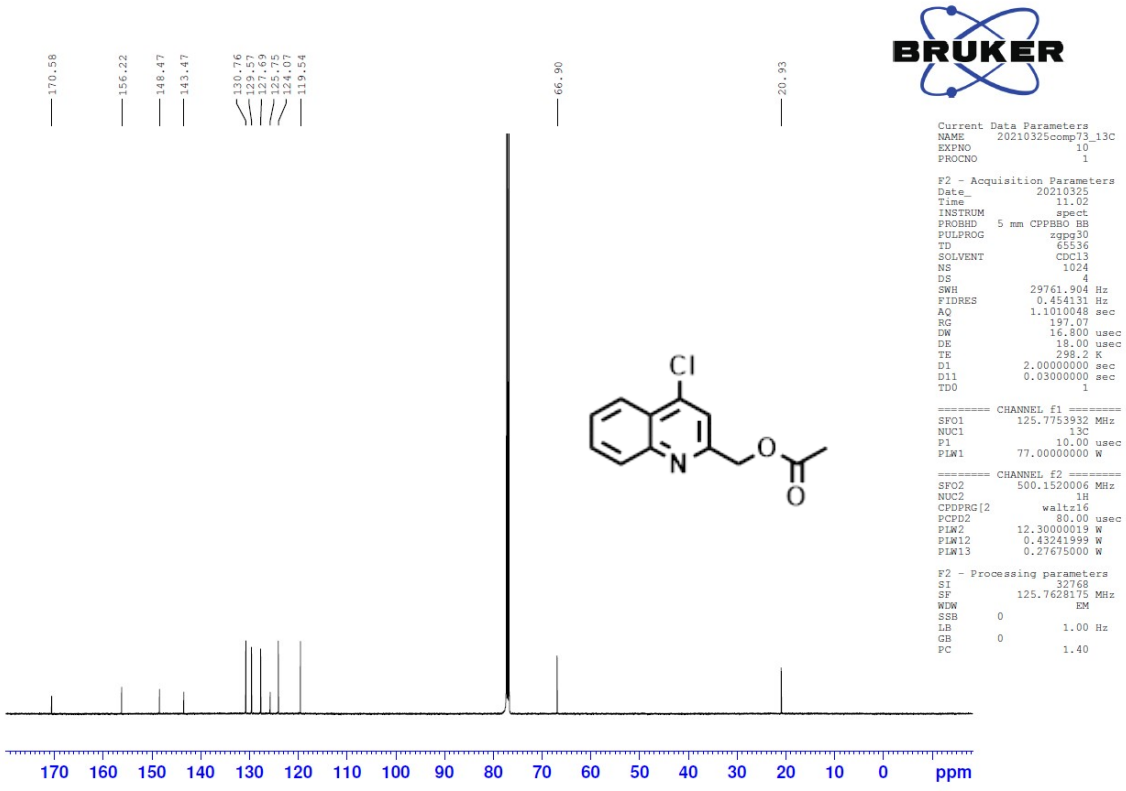


Figure S 47. ^{13}C NMR spectrum of compound 20 in $CDCl_3$.

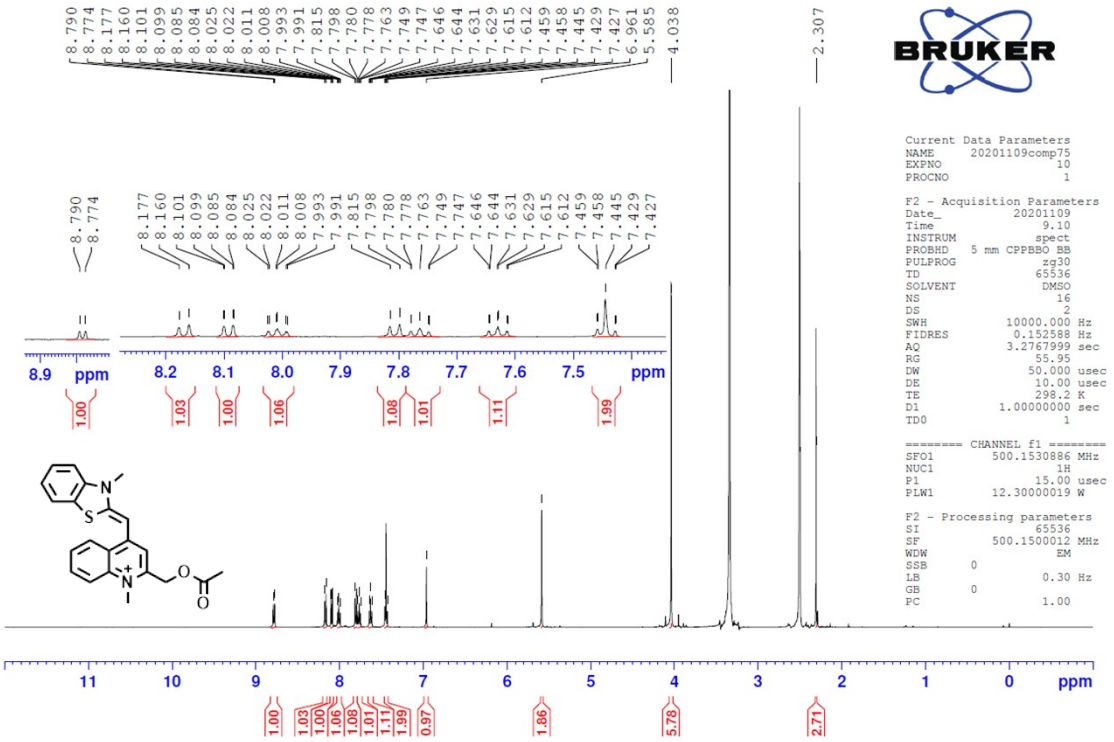


Figure S 48. ^{1}H NMR spectrum of compound 21 in DMSO-d_6.

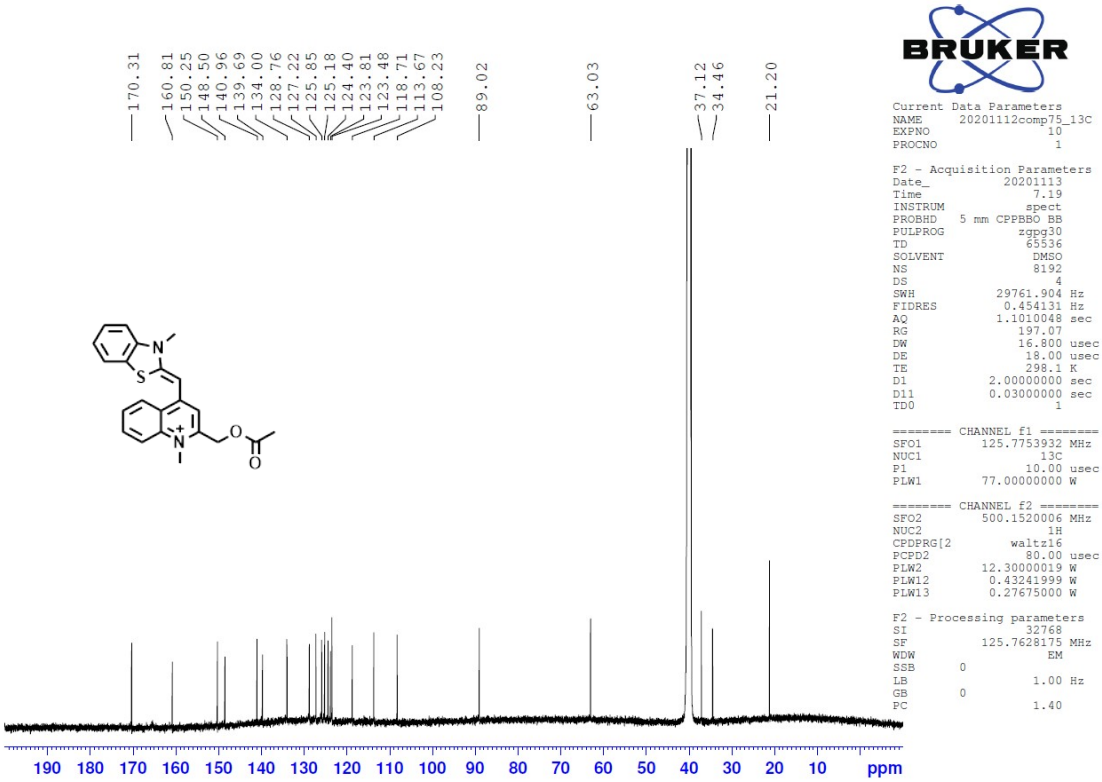


Figure S 49. ^{13}C NMR spectrum of compound 21 in DMSO-d_6.

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