SUPPORTING INFORMATION

Unambiguous Detection of Elevated Levels of Hypochlorous Acid in Double Transgenic AD Mouse Brain

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EXPERIMENTAL METHODS

General Methods. All reagents and solvents were obtained from Merck or Specrochem and used without further purification unless it is mentioned. The amyloid specific antibody (OC) for immunostraining was obtained from Merck (AB2286). All the moisture sensitive reactions were performed under nitrogen or argon atmosphere. Agilent Cary series UV−Vis-NIR absorption and Agilent Cary eclipse fluorescence spectrophotometers were used to perform absorption and fluorescence measurements, respectively. Origin 8.5 or Prism 6 software was used to process and analyze the raw data. NMR spectra (^{1}H and ^{13}C NMR) of all synthesized compounds were recorded by a Bruker AV−400 spectrometer (tetramethylsilane used as internal standard). HRMS spectra of all synthesized compounds were acquired on Agilent 6538 UHD HRMS/Q-TOF high-resolution spectrometer. The calculated amount of CM probes were dissolved in deionized water (Milli Q) (contain 5% dimethyl sulfoxide) and stored at -20 °C. Further, the stock solutions were diluted in phosphate buffered saline (PBS, pH= 7.4) for the experiments. The cells and the brain images were captured using confocal fluorescence microscope (Olympus FV3000).

Synthesis of ethyl-7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (I1). To a solution of *N*,*N*-diethylsalicyldehyde (2.5 g, 12.94 mmol) in EtOH (35 mL), diethylmalonate (2.36 mL, 15.52 mmol) and piperidine (1.5 mL) were added. The reaction mixture was refluxed at 85 °C for 12 h and monitored by thin layer chromatography (TLC). After completion of the reaction, solvent was evaporated under vacuum and the crude was diluted with water (30 mL) and the compound was extracted into dichloromethane (DCM) (3X 50 mL).The DCM extract was washed with water (1 X 25 mL), brine (1 X 30 mL), dried over Na_2SO_4 (anhydrous) and evaporated under vacuum. The crude peoduct was purified by column chromatography using hexane:ethyl acetate as eluent.
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H), 7.34 (d, *J*= 8.8 Hz, 1H), 6.59 (dd, *J*= 2.4 Hz, *J*= 6.8 Hz, 1H), 6.45 (d, *J*= 2.0 Hz, 1H), 4.36 (q, *J*= 7.2 Hz, 2H), 3.43 (dd, *J*= 7.2 Hz, *J*= 6.8 Hz, 4H), 1.38 (t, *J*= 7.2 Hz, 3H), 1.22 (t, *J*= 7.2Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.3, 157.4, 157.3, 151.8, 148.2, 130.0, 108.5, 107.9, 106.7, 95.7, 76.2, 76.0, 75.8, 60.1, 44.1, 13.4, 11.4. HRMS (ESI-MS): m/z calculated for $C_{16}H_{19}NO_4$ $[M+H]^+$ = 290.1387, observed 290.1395.

Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (I2). Ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (1.0 g, 3.41 mmol) was taken in a round-bottomed flask, conc. HCl (25 mL) was added dropwise, and the mixture was stirred at room temperature for 48 h. The reaction mixture was transfer into an ice bath and sodium hydroxide (10 M) was added dropwise into the reaction mixture, the precipitate formed was collected by suction filtration and dried under the vacuum.

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (s, 1H), 8.75 (s, 1H), 7.62 (s, *J*= 4 Hz, 1H) 6.77 (q, *J*= 12Hz, 1H), 6.55 (s, 1H), 3.47 (q, *J*= 24 Hz, 4H), 1.13 (t, *J*= 8 Hz, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.4, 159.6, 157.8, 152.8, 149.3, 131.7, 110.2, 107.1, 95.9, 44.3, 12.2. HRMS (ESI-MS): m/z calculated for $C_{14}H_{15}NO_4$ $[M+H]^+$ = 262.1001, observed 262.1046.

Synthesis and Characterization of CM1. To a solution of 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (0.5 g, 2.63 mmol) in DMF (10 mL), DIPEA (1.1 g, 10.52 mmol) HBTU (1.2 g, 3.15 mmol) and HOBt (0.42 g, 3.15 mmol) were added. The reaction mixture was allowed to stir for 15 min on the ice bath under nitrogen atmosphere and morpholine (275 mg, 3.15 mmol) was added, and the reaction mixture was further allowed to stir at room temperature for 12 h. After the completion of the reaction (monitored by TLC), DMF was evaporated under vacuum and the crude was diluted with water (30 mL), compound was extracted into DCM (3X 30 mL).The collected DCM extracts was washed with water (1 X 20 mL), brine (1 X 20 mL), dried over Na_2SO_4 (anhydrous) and evaporated under vacuum. The crude product was purified by column chromatography using hexane:ethyl acetate as eluent.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.99 (s, 1H), 7.50 (d, *J*= 8.9 Hz, H), 6.75 (dd, *J*= 8.9 Hz, 2.5 Hz, 1H), 6.56 (d, *J*= 2.3 Hz, 1H), 3.69-3.52 (m, 8H), 3.46 (dd, *J*= 14.0, 7.0 Hz, 4 H), 1.19-1.09 (m, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.1, 158.3, 156.6, 151.2, 143.9, 130.0, 115.7, 109.3, 107.1, 96.2, 66.2, 53.5, 47.1, 44.1, 41.8, 18.0, 16.7, 12.2. HRMS (ESI-MS): m/z calculated for $C_{18}H_{22}N_2O_4$ $[M+H]^+$ = 331.1652, observed 331.1649.

Synthesis and Characterization of CM2. To a solution of 7-(diethylamino)-3-(morpholine-4-carbonyl)-2H-chromen-2-one (100 mg, 3.89 mmol) in toluene (5 mL), Lawesson's reagent (157 mg, 3.89 mmol) was added and refluxed for 12 h. The solvent was evaporated under vacuum and the crude was diluted with water (20 mL), compound was extracted into DCM (3X 20 mL). The collected DCM extracts was washed with water (1 X 15 mL), brine (1 X 15 mL), dried over Na_2SO_4

(anhydrous) and evaporated under vacuum. The crude product was purified by column chromatography using hexane:ethyl acetate as eluent.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.96 (s, 1H,) 7.55 (d, J= 8.9 Hz, 1H,) 6.78 (dd, J= 8.9 Hz, 2.4 Hz, 1H), 6.60 (d, J= 2.3 Hz, 1H), 4.41-4.33 (m, 1H), 4.21 (m, 2H), 3.81-3.74 (m, 2H), 3.49 (q, J= 7.1 Hz. 4H), 1.16 (t, J= 7.0 Hz, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 191.8, 157.3, 156.3, 151.0, 143.1, 130.3, 121.9, 109.4, 107.3, 96.2, 66.1, 65.6, 52.2, 49.3, 44.1, 12.2. HRMS (ESI-MS): m/z calculated for $C_{18}H_{22}N_2O_4$ $[M+H]^+$ = 347.1424, observed 347.1434.

Absorption and Fluorescence Spectroscopy. UV-visible absorption and fluorescence spectroscopy measurements were carried out using single beam Agilent Cary series UV−Vis-NIR absorption and Agilent Cary eclipse fluorescence spectrophotometers at room temperature, respectively. 1 cm path length quartz cuvette (1 mL) was used for all the absorption and fluorescence measurements.

Preparation of Reactive Oxygen Species (ROS). Various ROS (NO^{2-}, NO^{3-}, NO, t-BuOOH, H_2O_2, $HO^{\cdot}$, $^1O_2$ and OCl^-) were prepared according to the following protocols. Calculate amount of $NaNO_2$, $NaNO_3$ and sodium nitroferricyanide(III) dihydrate were dissolved in PBS (pH= 7.4, 10 mM) to obtain NO^{2-}, NO^{3-} and NO, respectively. Analytical grade t-BuOOH and H_2O_2 were obtained from Alfa Aesar and used as received. Hydroxyl radicals were generated *in situ* using Cu^{II}-Ascorbate redox pair. Singlet oxygen was generated using NaOCl and H_2O_2. NaOCl was used to generate ClO^- in situ and its concentration was measured using absorption study (ε= 350 M^{-1} cm^{-1} at 292 nm) at pH 9.0. The stock solutions of metal ions (Ni^{II}, Zn^{II}, Ca^{II}, Co^{II}, Na^+, K^+, Al^{III}, Fe^{III}, Cd^{II} and Pb^{II}) were prepared by dissolving calculated amounts of $NiCl_2$, $ZnCl_2$, $CaCl_2$, $CoCl_2$, NaCl, KCl, $AlCl_3$, $FeCl_3$, $CdSO_4$ and $PbNO_3$ salts, respectively, in deionized water (Milli Q). Similarly, the stock solutions of anions (Asc, $S_2O_8^{2-}$, S^{2-}, N_3^-, Cl^-, I^-, CO_3^-, OAc^-, AsO_4^{3-}, SO_4^{2-} and $S_2O_3^{2-}$) were prepared by dissolving calculated amounts of sodium ascorbate, $Na_2S_2O_8$, Na_2S, NaN_3, NaCl, KI, Na_2CO_3, NaOAc, Na_3AsO_4, Na_2SO_4 and $Na_2S_2O_3$, respectively, in deionized water (Milli Q).

Response of CM2 in Presence of Aβ42. Response of **CM2** was evaluated by measuring the fluorescence in presence of freshly prepared monomers (m), oligomers (o), and fibrils (f) of Aβ42. Aβ42 peptide (0.25 mg, Calbiochem, Merck) was dissolved in HFIP (200 µL) and sonicated for 30 min. Aβ42 sample was dried under the nitrogen flow and further dissolved in PBS (pH 7.4, 50

mM) containing NaCl (150 μM). The Aβ42 solution was incubated to produce oligomers and fibrils at 4 °C and 37 °C, respectively. Finally, **CM2** (10 μM) was added to different forms (monomers, oligomers, and fibrils) of Aβ42 (10 μM) and fluorescence (λ_{ex} = 417 nm and λ_{em} = 485 nm) was measured using microplate reader (SpectrsMax i3x).

Cell Culture. SHSY5Y cells were cultured using DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) medium (Gibco, Invitrogen) containing 10% of FBS (fetal bovine serum) and 1% PS (pen-strep) under the cell growing condition (37 °C temperature and 5% CO_2 atmosphere). For imaging experiments the cells were cultured in 35 mm petri dish under similar condition and the cells were fixed using 4% paraformaldehyde (PFA) solution.

Cytotoxicity Assay. To evaluate the cytotoxicity of **CM2**, we performed MTT assay in SHSY5Y cells. The cells were (15,000 cells/well) cultured in a 96-well plate using cell growing media and incubated with different concentrations of **CM2** for 24 h. Further MTT (15 mL, 5 mg/mL) solution was added into the cell media and incubated for 3 h. Finally, the medium was exchanged with 100 μL DMSO-MeOH (1:1) mixture and the absorbance was monitored at 570 nm using microplate reader (SpectraMax i3x, Molecular Devices).

Intracellular HOCl Imaging. To demonstrate the ability of **CM2** for the detection and imaging of HOCl in cellular conditions, we performed imaging experiments with SHSY5Y cells. For this experiment, SHSY5Y cells were cultured in 35 mm petri dish with cell growing media. The cells were fixed with 4% PFA and incubated with **CM2** (0.5 μM) and DAPI for 30 min. The cells were washed three times with PBS and again incubated with NaOCl (400 μM) for 30 min. Finally, the cells were washed and imaged under the confocal fluorescence microscope.

Genotyping of Mice. The mice were genotyped after attaining the age of weaning at 4-6 weeks. To collect genomic DNA, the tail was collected from each and every mouse and processed with NaOH and Tris-HCl buffer. The genotyping was performed according to the Jackson Laboratory's protocol and mice were confirmed as Alzheimer's positive or WT mice. The primer sequences used for genotyping are as follows.

APP: 5'-GACTGACCACTCAGCCAGGTTCTG-3', 5'CTTGTAAGTTGGATTCTCATATCC G-3'; PSEN1: 5'-ATTAGAGAACGGCAGGAGCA-3', 5'-GCCATGAGGGCACTAATCAT-3'.

Blood Brain Barrier (BBB) Crossing Experiment. For this BBB experiment, PBS (control) and **CM2** were administrated to WT C57BL/6 mice and sacrificed after 45 and 90 min to collect the brains. Then the brains were homogenized with equal amount (1.5 mL) of PBS and supernatants were collected for spectroscopy study. The absorbance and fluorescence of brain supernatants were measured in microplate reader in the absence and presence of OCl^-. All the data were plotted and analyzed in prism 6 software. Next, dichloromethane (DCM) extract of the brain lysate was further concentrated for mass analysis (MALDI) using α-cyano-4-hydroxycinnamic acid (CCA) matrix.

Image Analysis. All the brain images were analyzed and the intensity of green and red fluorescence was calculated using ImageJ software. GraphPad Prism was used to plot and analyze the results. Two-way ANOVA was used to analyze datasets with more than one independent variable. Further, Bonferroni's multiple comparisons Post hoc test was applied to determine the significant difference in the result ($P < 0.05$).

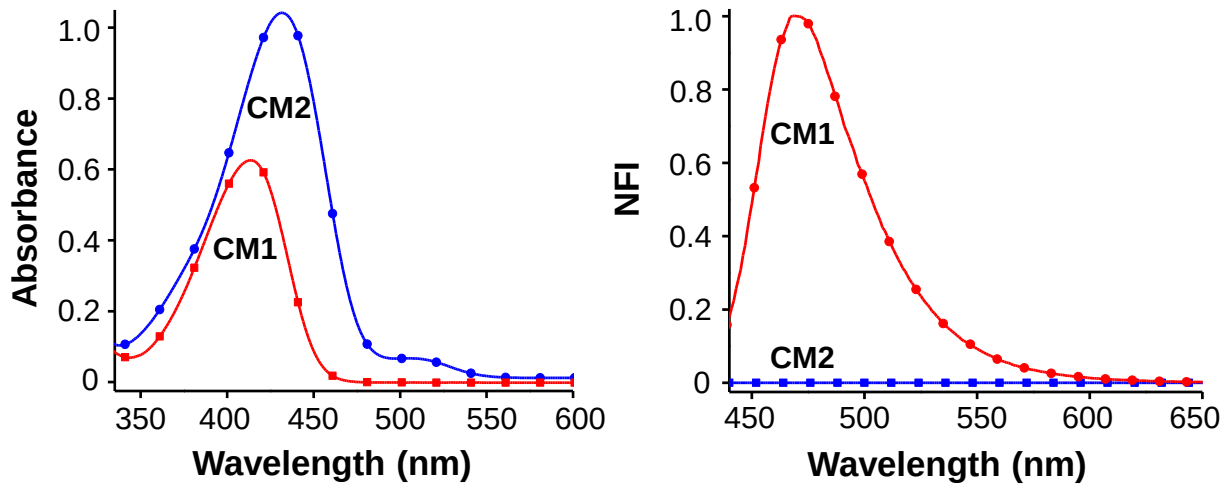


Figure S1. The absorbance and fluorescence spectra of **CM1** and **CM2**. NFI: Normalized fluorescence intensity.

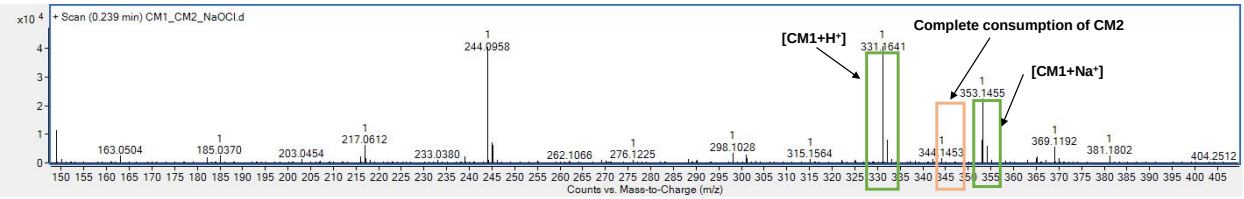


Figure S2. High resolution mass spectrometry (HRMS) analysis of **CM2** in presence of NaOCl.

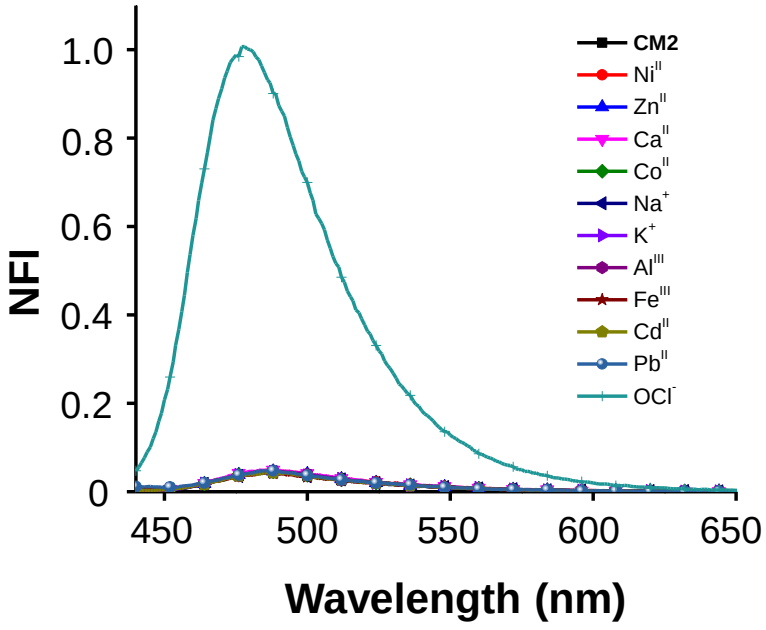


Figure S3. The fluorescence spectra of **CM2** in presence of OCl^- and various metal ions. NFI: Normalized fluorescence intensity.

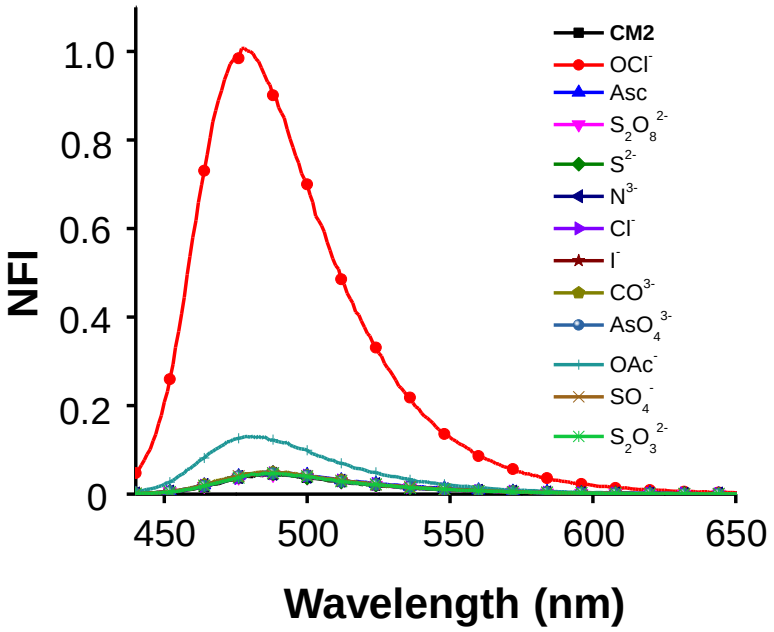


Figure S4. The fluorescence spectra of **CM2** in presence of OCl^- and various anions. NFI: Normalized fluorescence intensity.

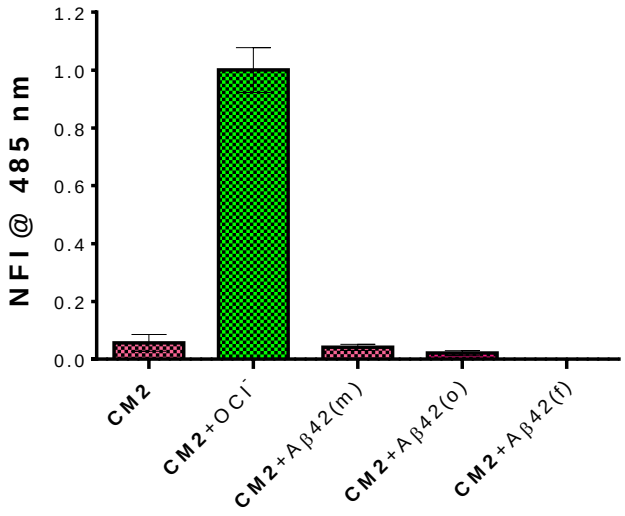


Figure S5. Fluorescence intensity of **CM2** in presence of monomers (m), oligomers (o), and fibrils (f) of Aβ42. External NaOCl was used as a positive control. NFI: Normalized fluorescence intensity.

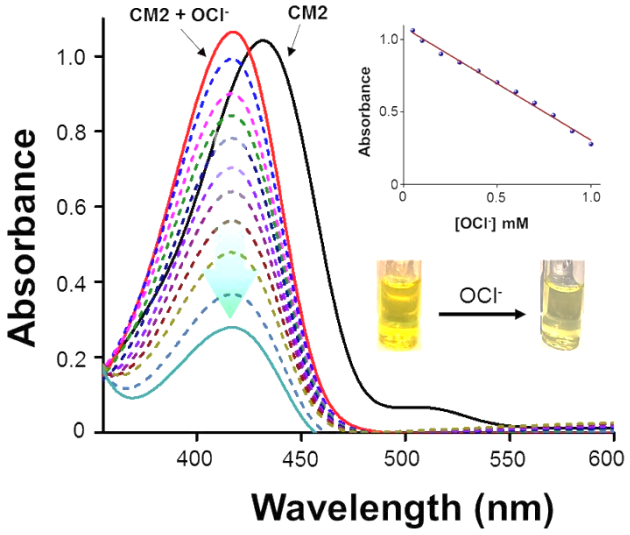


Figure S6. The absorption spectra of **CM2** with increasing concentrations of OCl^- (Insets: change in absorbance maxima (λ_{max}= 417 nm) with OCl^-concentrations and colorimetric change of **CM2** solution from yellowish to colorless).

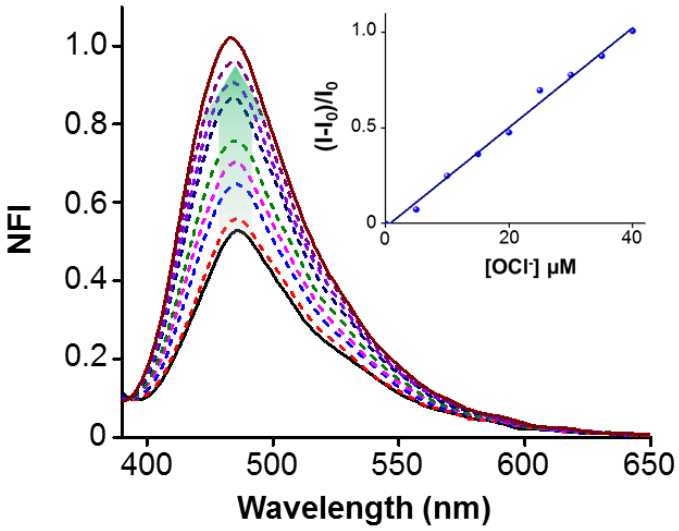


Figure S7. The fluorescence spectra of **CM2** in presence of very low concentration (5 – 40 μM) of NaOCl. NFI: Normalized fluorescence intensity.

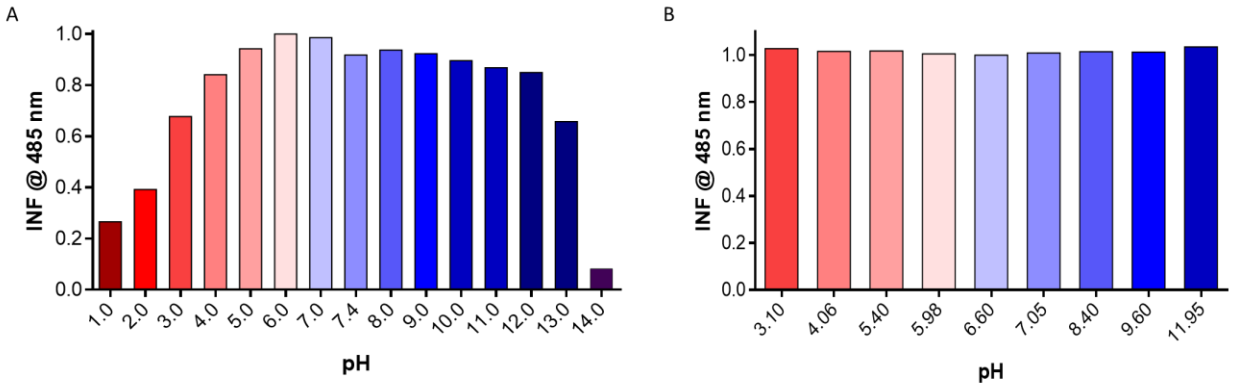


Figure S8. Normalized fluorescence intensity (NFI) of **CM2** with NaOCl at different pH in PBS (A) and Milli Q water (B).

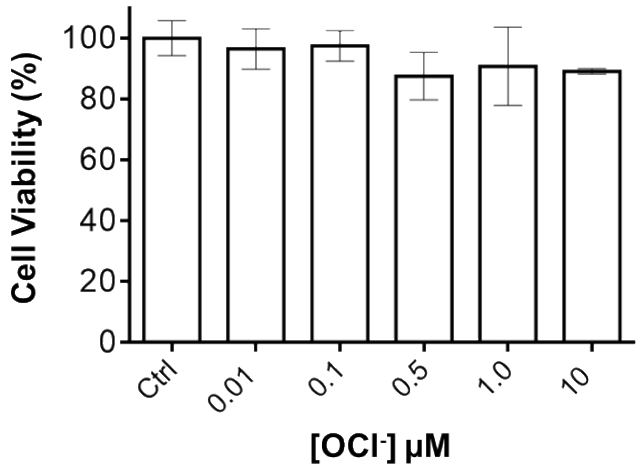
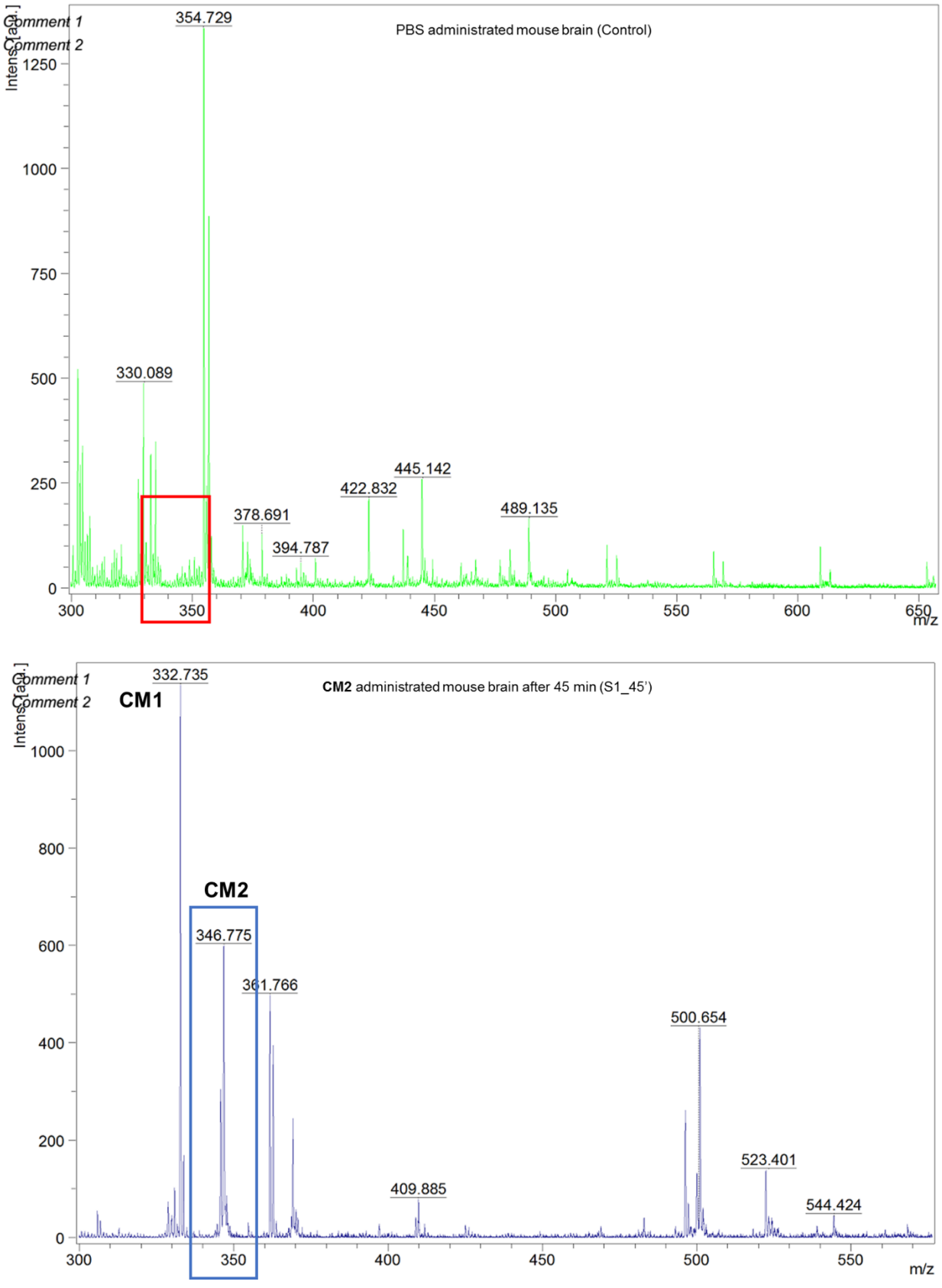


Figure S9. Cytotoxicity study of **CM2** at different concentrations in SHSY5Y cells.

Figure S10. MALDI mass analysis of brain lysates.



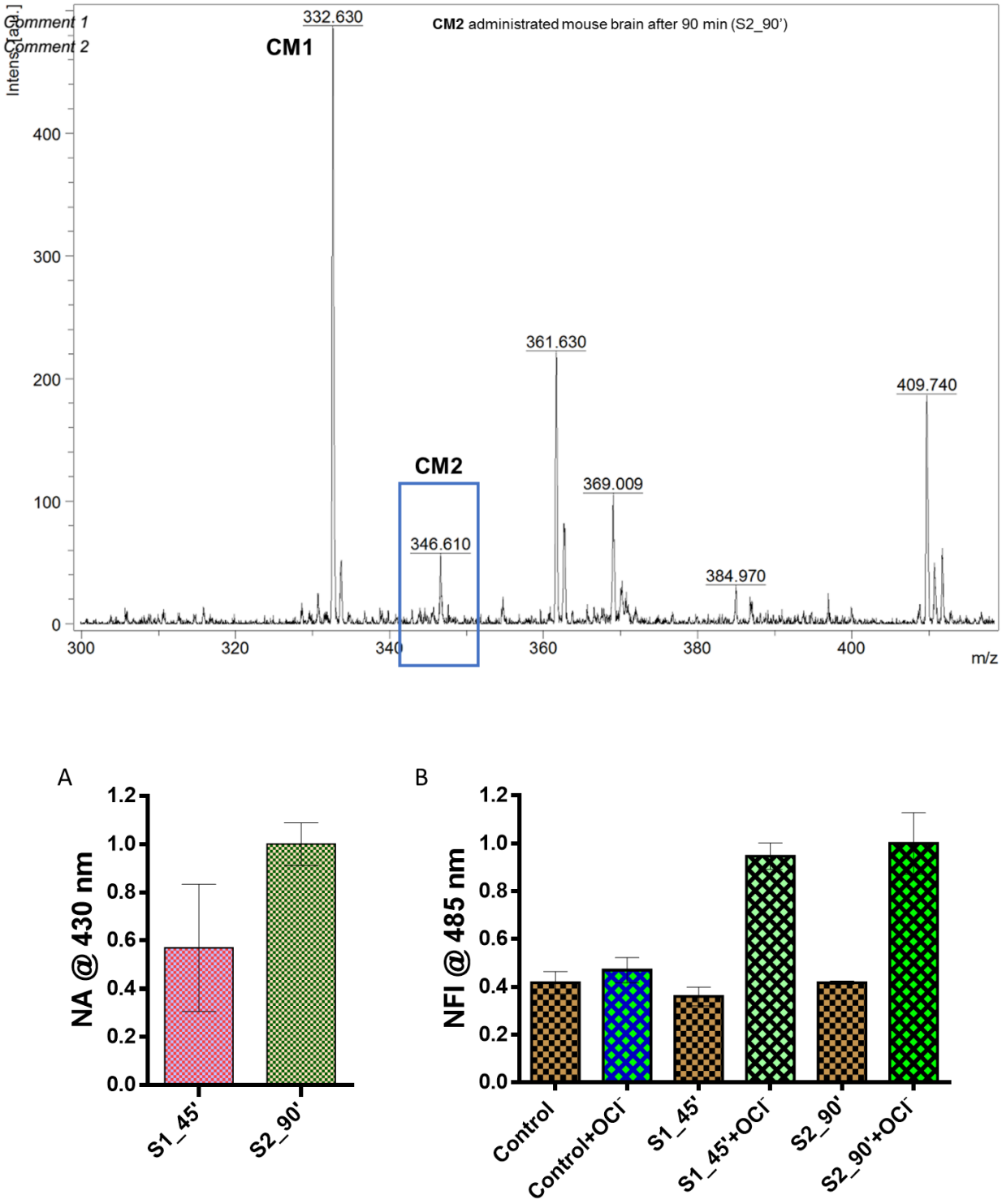
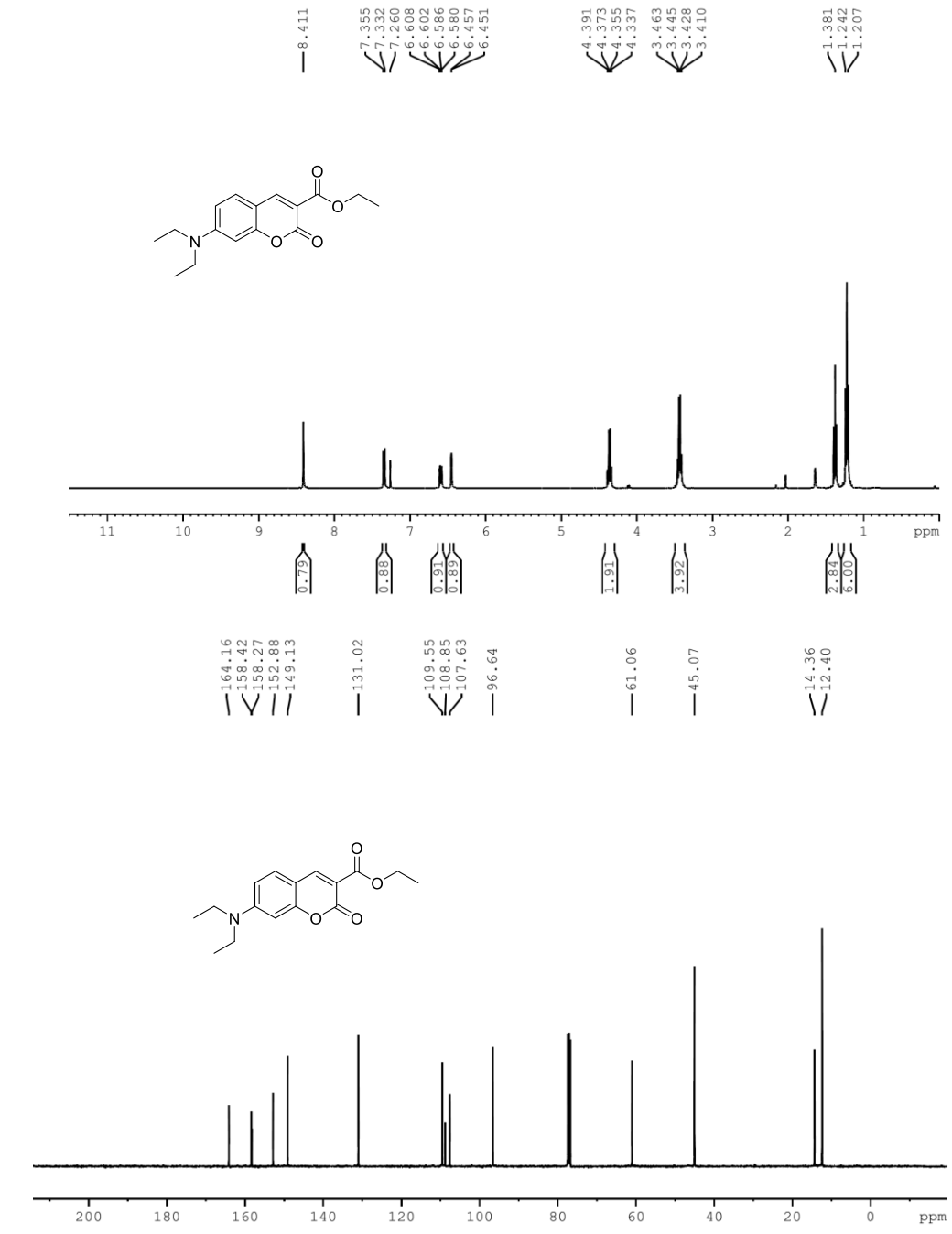


Figure S11. A) The normalized absorbance (NA) of **CM2** administrated brain samples collected after 45 (S1_45') and 90 (S2_90') min of probe administration. The presented data is baseline corrected with PBS administrated brain sample (control). B) Normalized fluorescence intensity (NFI) of PBS (control) and **CM2** administrated brain samples in absence and presence of OCl^- (λ_{ex}= 417 nm).

Crystallographic data of CM2 (CCDC Deposition Number 1955148)

Crystallographic Parameters	Crystallographic Data
Formula	$C_{18}\ H_{22}\ N_2\ O_3\ S$
Formula weight	346.43
Crystal system	Monoclinic
Space group	*C c*
a (Å)	13.3839(5)
b (Å)	19.2766(9)
c (Å)	8.6173(3)
α (°)	90
β (°)	126.480
γ (°)	90
Z	4
F(000)	736
Temperature (K)	296(2)
V (Å^3)	1787.62(13)
Calculated density (μg·m^{-3})	1.287
Crystal size (mm^3)	0.48X 0.23 X 0.12
μ (mm^{-1})	0.199
Limiting indices	-17≤ h ≤ 17, -25 ≤ k≤ 25, -11≤ l ≤ 9
Reflections collected/ Unique	21010/ 3467
R_1, wR_2 [all data]	0.0790, 0.1875
R_1, wR_2 [$I > 2\sigma(I)$]	0.0641, 0.1718
Largest diffraction peck and hole (e Å^{-3})	0.806/ -0.272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ppm

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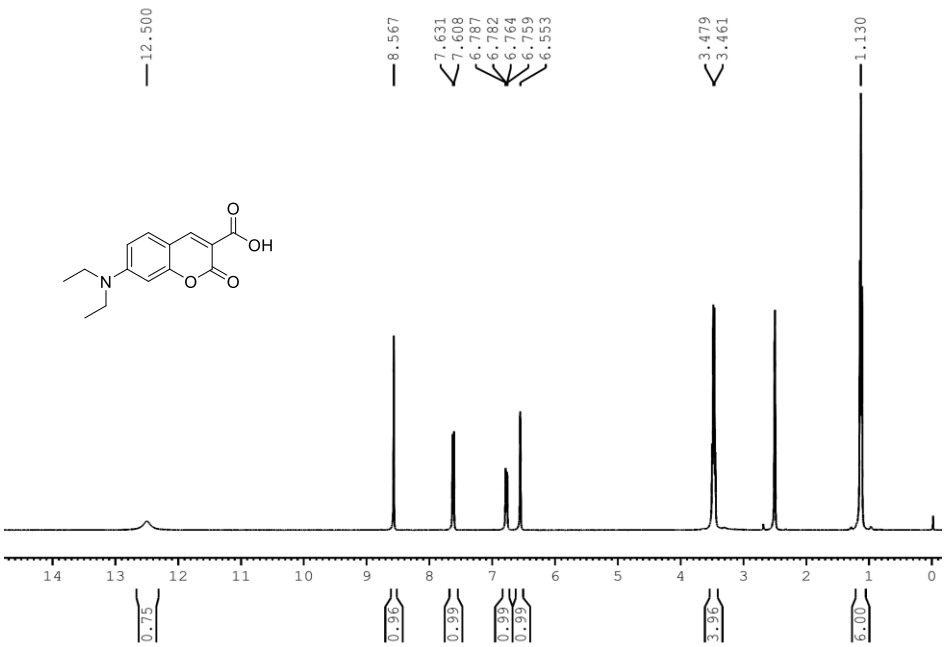
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OH
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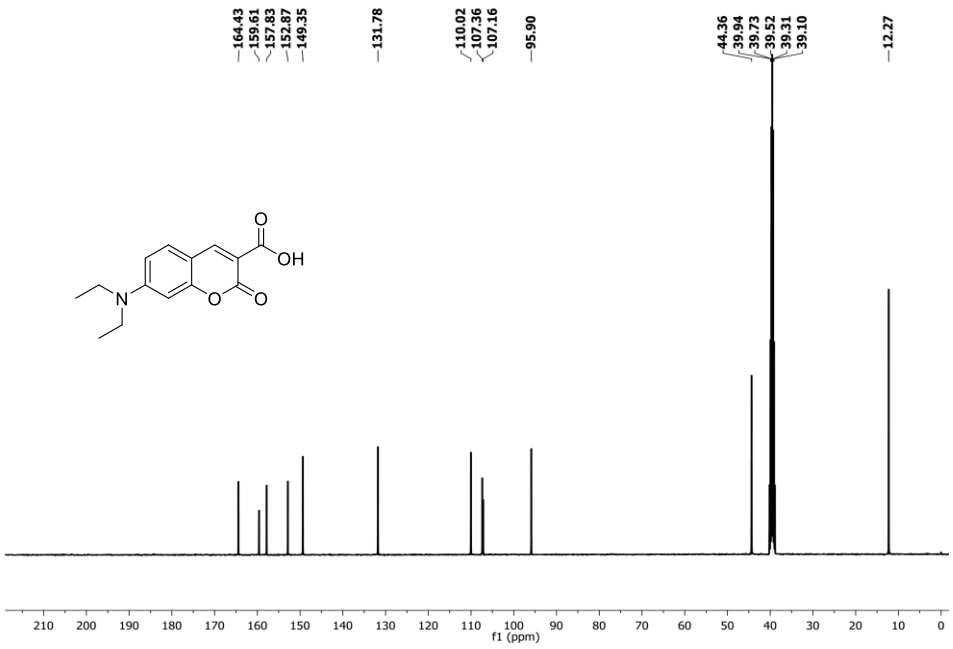
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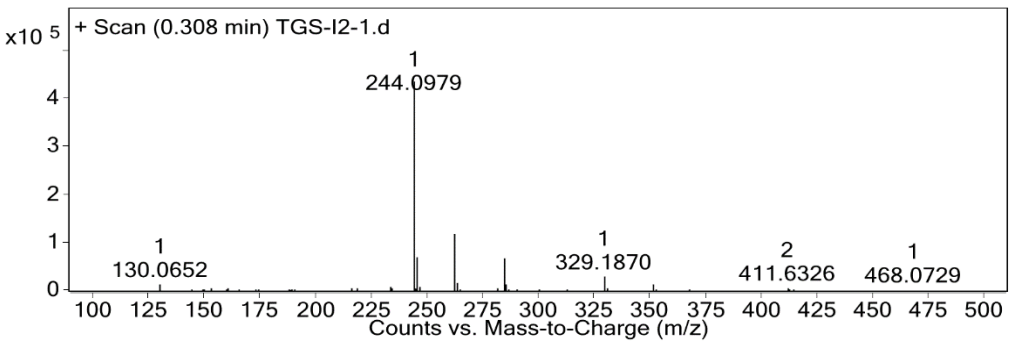
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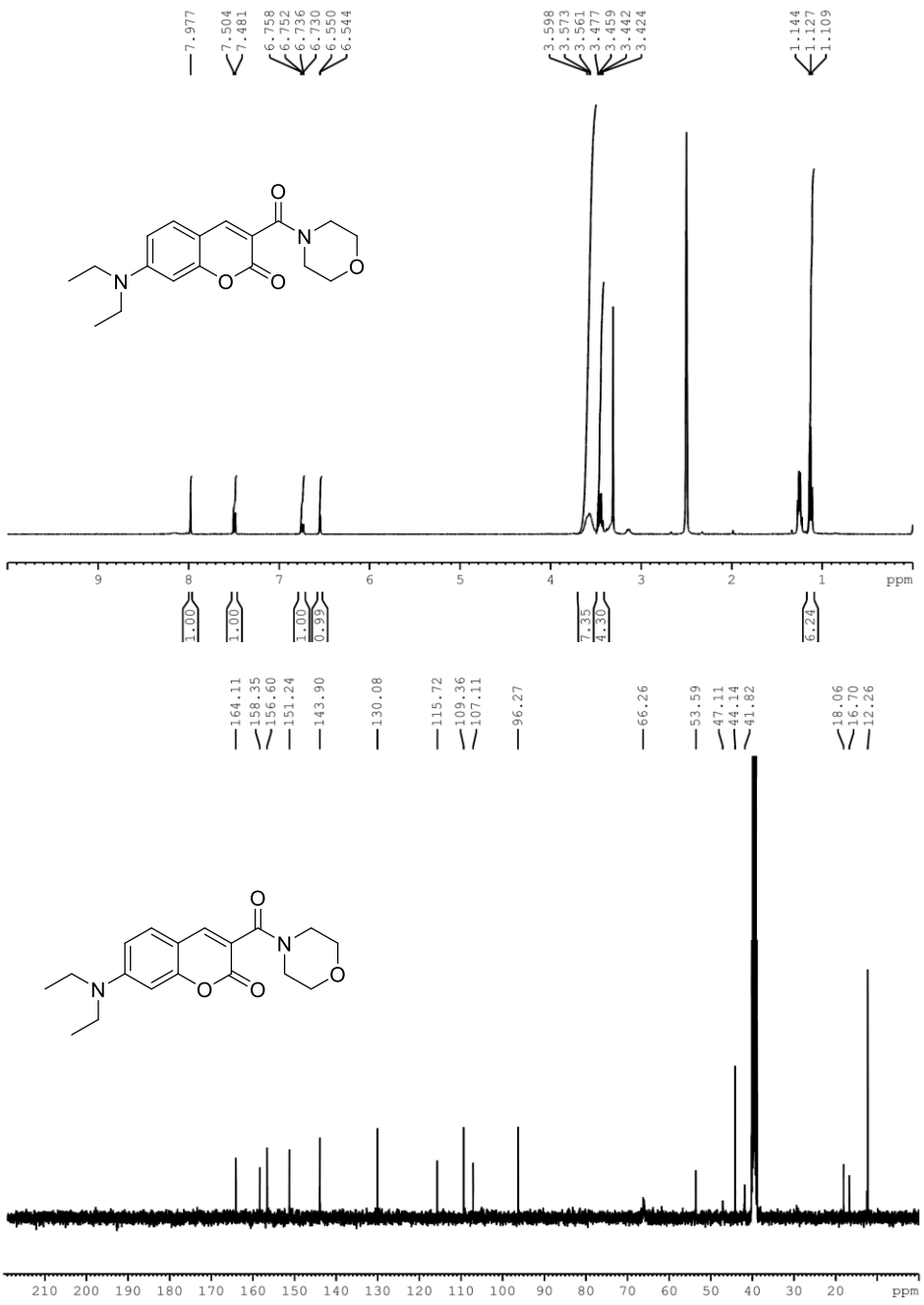
Counts vs. Mass-to-Charge (m/z)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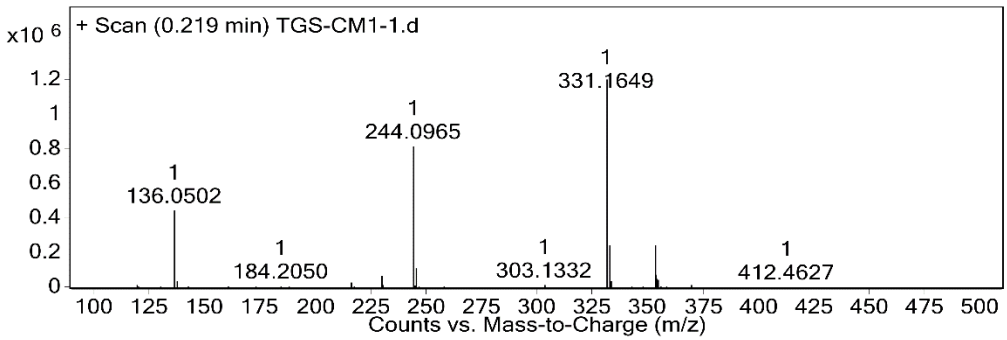
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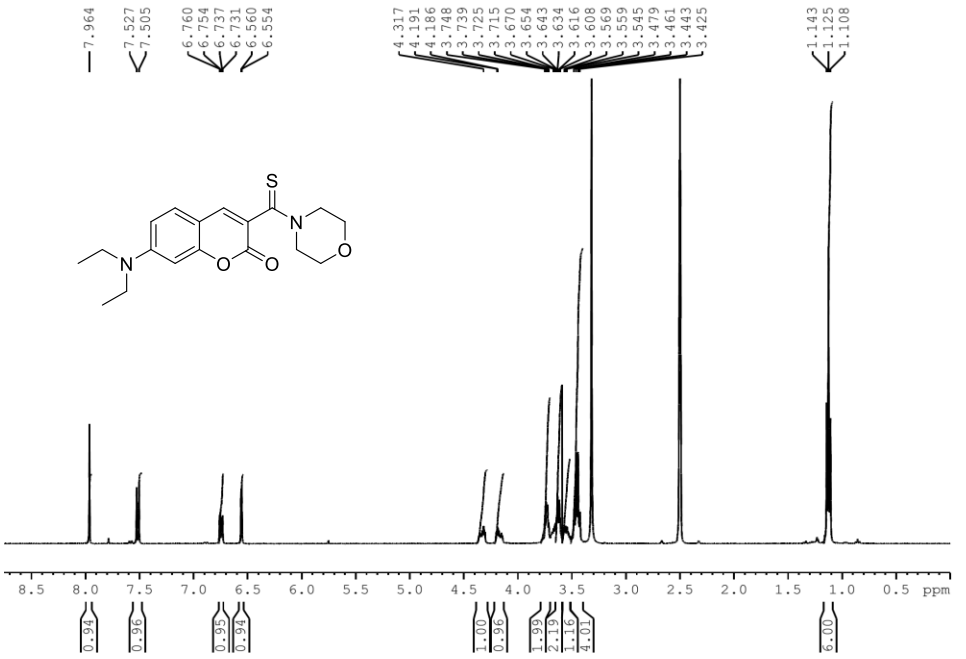
Counts vs. Mass-to-Charge (m/z)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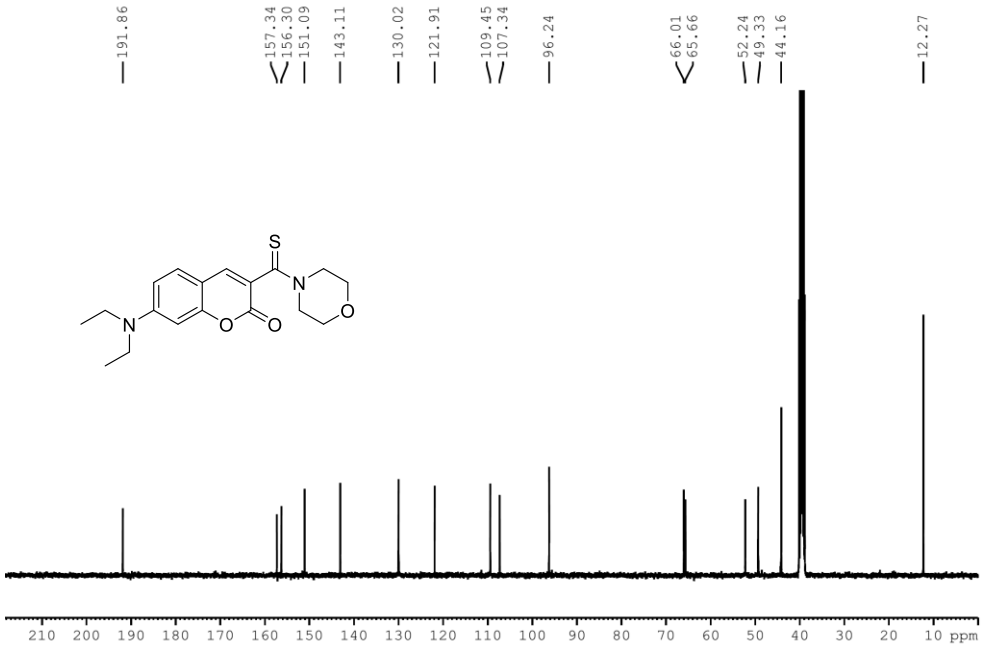
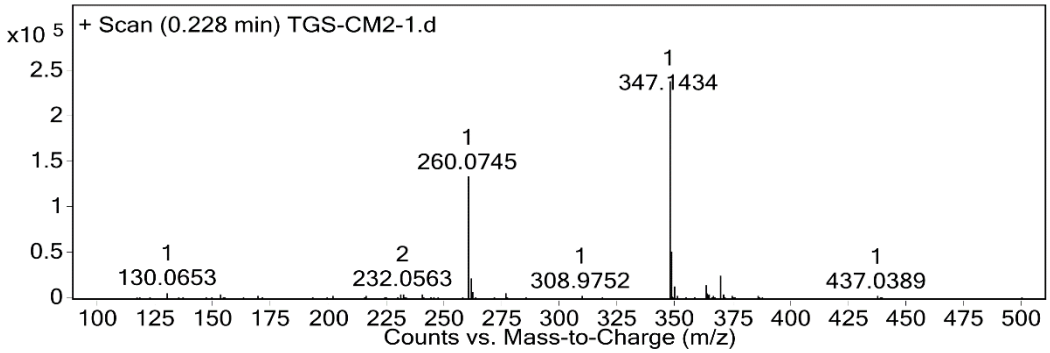
3.559
3.545
3.479
3.461
3.443
3.425
1.143
1.125
1.108
S
N
O
O
N
O
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.94
0.96
0.95
0.94
1.00
0.96
1.99
2.19
1.16
4.01
6.00


191.86
157.34
156.30
151.09
143.11
130.02
121.91
109.45
107.34
96.24
66.01
65.66
52.24
49.33
44.16
12.27
S
N
O
O
N
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


x10 5
+ Scan (0.228 min) TGS-CM2-1.d
2.5
2
1.5
1
0.5
0
1
347.1434
1
260.0745
1
130.0653
2
232.0563
1
308.9752
1
437.0389
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
Counts vs. Mass-to-Charge (m/z)