

Electronic Supplementary Information

3-Benzoylquinoxalinone, as photoaffinity labelling derivative, with fluorogenic properties allowing reaction monitoring under “no-wash” conditions

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I. General

General Information

All chemicals were used as received from commercial sources without further purification. Solvents, unless otherwise stated, were purchased in reagent grade or HPLC grade and used as received. PBS (pH 7.4, 0.1 M), Tris Buffer (pH 7.6, 0.05 M) and aq. mobile phases for HPLC were prepared with water that was purified by means of a MilliQ system (purified to 18.2 M Ω cm). CA-II (carbonic anhydrase isozyme II from bovine erythrocytes, $\geq 95\%$ (SDS-PAGE), specific activity $\geq 3,500$ units/mg protein, lyophilized powder, were purchased from Sigma-Aldrich. Human blood plasma (HBP) was furnished by the Centre Hospitalier Universitaire de Rouen and was collected under medical conditions and supervisions from unknown donors. (CAUTION! HBP is a biological substance that must be handled with care by trained personnel. Vials, needles, pipettes, etc. must be considered and treated as biological wastes). All reactions were monitored by thin layer chromatography (TLC). TLC were carried out on Merck DC Kieselgel 60 F-254 aluminum sheets. Visualization of spots was performed under a UV lamp at $\lambda = 254$ or 365 nm, and/or staining with a KMnO₄ solution/K₂CO₃ + 5% NaOH, developed with heat. Flash column chromatography purifications were performed manually on silica gel (40–63 μ m) under pressurized air flow.

Instruments and methods

¹H, ¹³C spectra were recorded on 300 MHz Bruker FT-NMR machine operating at ambient probe temperature. The solvent resonance was used as the internal standard for ¹H-NMR (chloroform-d at 7.26 ppm; DMSO-d₆ at 2.50) and ¹³C-NMR (CDCl₃ at 77.0 ppm; MeOH-d₄ at 49.0 ppm; DMSO-d₆ at 39.5). Chemical shift (δ) were quoted in parts per million (ppm). Coupling constants (J) were quoted in Hertz (Hz). The following abbreviations were used to give the multiplicity of the NMR signals: s: singlet, bs: broad singlet, d: doublet, t: triplet, dd: doublet of doublet...

High Resolution Mass spectrometry (HRMS) was performed using a Waters Micromass LCT Premier XE[®] equipped with an orthogonal acceleration time-of-flight (oa-TOF) and an electrospray source in positive or negative mode.

UV-Vis spectroscopy was performed on a Agilent Cary 60 UV-Vis[®] and was performed using a quartz fluorescence cell (Hellma, 104F-QS, 10 \times 10 mm, pathlength 10 mm, chamber volume 3.5 mL). Fluorescence spectroscopic studies (emission/excitation spectra) were performed on a Fluorolog-3-21 (Horiba) spectrophotometer using a quartz fluorescence cell (Hellma, 104F-QS, 10 \times 10 mm, pathlength 10 mm, chamber volume 3.5 mL). The samples were irradiated by a continuous 450 W Xe lamp, equipped with a double 330 nm grated monochromator and the luminescence was collected at 90 $^\circ$ through a simple 500 grated monochromator and measured by a Hamamatsu photomultiplier tube R13456. The O.D. of the samples was kept below 0.1 to avoid inner filter effect and self-quenching. A correction factor was applied to take account of excitation fluctuation and detector and grating spectral response.

Emission spectra of *in vitro* photoaffinity labelling of CAII were performed without dilution using an ultra-micro quartz cell (Hellma, 105.251-QS, 20 × 3 mm, pathlength 3 mm, chamber volume 45 µL).

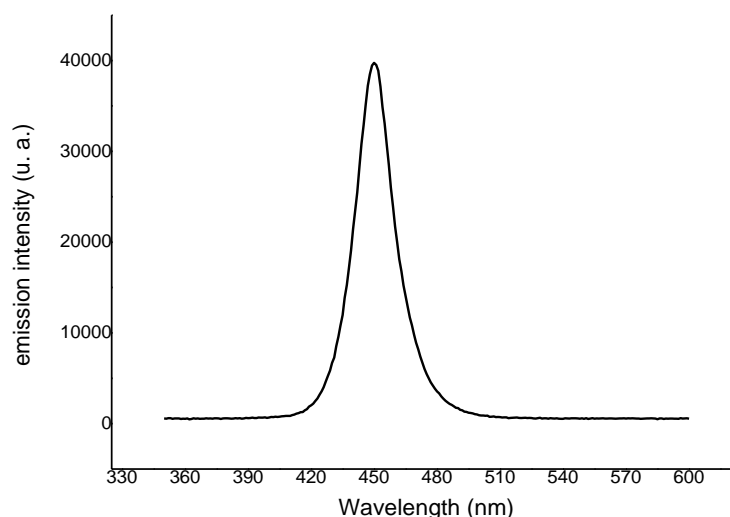
The absorption spectra of compounds were recorded (250–700 nm) at 25 °C. Excitation/emission spectra were recorded under the same conditions after emission/excitation at the corresponding wavelength depending on fluorophores (excitation and emission filters: auto, excitation and emission slit: 2 or 5 nm). Fluorescence quantum yields were measured at 25 °C by a relative method using Quinine Sulfate 350 ($\Phi_F = 0.55$ in sulfuric acid solution 0.5 M)¹ with excitation at 366 nm and Lucifer Yellow ($\Phi_F = 0.21$ in water)² with excitation at 366 nm as a standard. The following equation was used to determine the relative fluorescence quantum yield:

$$\Phi_F (X) = (A_s/A_x) (F_x/F_s) (n_x/n_s)^2 \Phi_F (S)$$

where A is the absorbance (in the range of 0.01–0.1 a.u.), F is the area under the emission curve, n is the refractive index of the solvents (at 25 °C) used in measurements, and the subscripts s and x represent standard and unknown, respectively. The following refractive index values were used: 1.337 for PBS, 1.333 for water and 1.339 for sulfuric acid solution.

Photoreactions were performed with different LED light sources:

1) A visible blue LED strip light (JnDee Blue 1M, 60 blue LED SMD 3528 (λ_{\max} 450 nm), 12 Volts DC, 5 Watts). Its emission spectrum was determined on a Fluorolog-3-21 (Horiba) spectrophotometer:



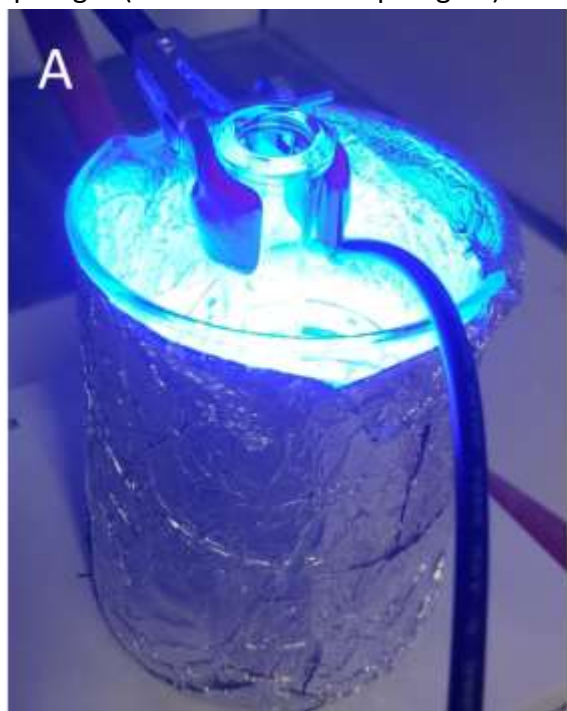
2) A visible blue LED spotlight (EvoluChem™ LED spotlights, 450PF (λ_{\max} 450 nm), 18 Watts).

3) A near UV LED spotlight (EvoluChem™ LED spotlights, 405PF (λ_{\max} 405 nm), 18 Watts).

4) A UV LED spotlight (EvoluChem™ LED spotlights, 365PF (λ_{\max} 365 nm), 18 Watts).

All emission spectra of EvoluChem™ LED spotlights are accessible online <https://www.hepatochem.com/photoreactors-leds-accessories-old/led-evoluchem/>

Photographs of labelling experiments with: A/ visible blue LED strip light (450 nm); B/ LED spotlight (EvoluChem™ LED spotlights)



RP-HPLC analyses were performed with a Thermo Scientific Ultimate® 3000 RS instrument, equipped with a diode array detector (DAD-3000RS) and temperature of the column compartment was fixed at 25 °C. A Thermo Fisher Hypersyl GOLD® column (1.9 µm, 2.1 × 50 mm) was used with a binary solvent system composed of MeCN and 0.1% aq. formic acid (aq. FA, pH 2) as eluents (linear gradient from 5 to 100% MeCN over 6 min; 100% MeCN for 1.5 min; linear gradient from 100 to 5% MeCN over 1.5 min; 5% MeCN for 2 min) at a flow rate of 0.600 mL/min.

RP-HPLC-MS analyses were performed with a Thermo Scientific Vanquish® (binary pump system) and a Thermo Scientific ISQ® EC instruments, equipped with a Waters BEH C18 column (1.7 µm, 2.1 × 100 mm). Temperature was maintained to 40 °C. A PDA detector was coupled with a low-resolution MS detector (ESI⁺). Solvent system was composed of MeCN and 0.1% aq. formic acid (aq. FA, pH 2) as eluents (linear gradient from 5 to 100% MeCN over 10 min; 100% MeCN for 1.5 min; linear gradient from 100 to 5% MeCN over 0.5 min; 5% MeCN for 3 min) at a flow rate of 0.500 mL/min.

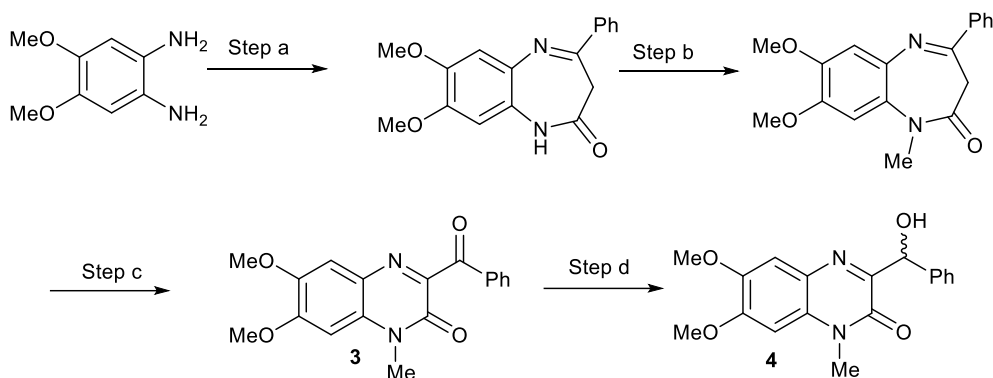
Semi-preparative RP-HPLC were performed with a Thermo Scientific Spectra SYSTEM SCM1000/P4000 equipped with a UV-visible detector, and a Thermo Scientific Synchronis Aq column (5 µm, 20 × 250 mm) with MeCN and 0.1 % aq. FA as eluents (95 % FA for 5 min, linear gradient from 5 to 100 % MeCN over 32 min) at a flow rate of 15 ml/min.

CA-II inhibitory activity assay were performed with a Spark® microplate reader (TECAN) using a 96-well plates. Absorbance was recorded through a light source dedicated xenon flash lamp.

II. Synthesis

Quinoxalinone derivatives **1**, **2**³ were prepared according to known procedures.

Preparation of quinoxalinones **3** and **4**



4,5-Dimethoxybenzene-1,2-diamine hydrochloride is commercially available, but the hydrochloride-free diamine could also be prepared from 4,5-dimethoxy-1,2-dinitrobenzene with the following procedure: To a solution of 4,5-dimethoxy-1,2-dinitrobenzene (0.500 g, 2.19 mmol) in 20 mL of a mixture EtOAc/MeOH (6/4) was added Pd/C (0.233 g, 0.219 mmol, 0.1 equiv) under H₂ atmosphere. The mixture was stirred overnight at room temperature. After completion the mixture was filtered under Celite®, and washed with EtOAc. The product was obtained in quantitative yield as a black solid. The analysis data is consistent with literature.³

7,8-dimethoxy-4-phenyl-1,3-dihydro-2H-benzo[b][1,4]diazepin-2-one (Step a). To a stirred boiling solution of 4,5-dimethoxybenzene-1,2-diamine (0.500 g, 2.97 mmol) in *p*-xylene (7 mL), ethyl 3-oxo-3-phenylpropanoate was added (0.686 g, 3.57 mmol, 1.2 equiv) dropwise and refluxed for 3 h. The reaction mixture was left to stand at room temperature for 24 h. The precipitated solid was collected by filtration and recrystallized from ethanol to give the desired product 7,8-dimethoxy-4-phenyl-1,3-dihydro-2H-benzo[b][1,4]diazepin-2-one (0.518 g, 1.75 mmol, 59% yield) as a white solid, mp = 130 °C. IR (neat): 3675, 3207, 3092, 2965, 2910, 2833, 1664, 1509, 1250, 1238, 1037, 1023. ¹H NMR (300 MHz, DMSO): δ 10.33 (s, 1H), 8.04 (dd, J = 6.8, 3.0 Hz, 2H), 7.59 – 7.44 (m, 3H), 6.93 (s, 1H), 6.74 (s, 1H), 3.80 (s, 1H), 3.78 (s, 1H), 3.46 (s, 1H). ¹³C NMR (75 MHz, DMSO): δ 165.3, 155.8, 147.4, 145.7, 137.5, 133.0, 130.6, 128.7, 127.4, 123.4, 109.9, 104.5, 55.6, 55.6. HRMS (ESI+) Calculated for C₁₇H₁₇N₂O₃ [M+H]⁺: 297.1239; found: 297.1237.

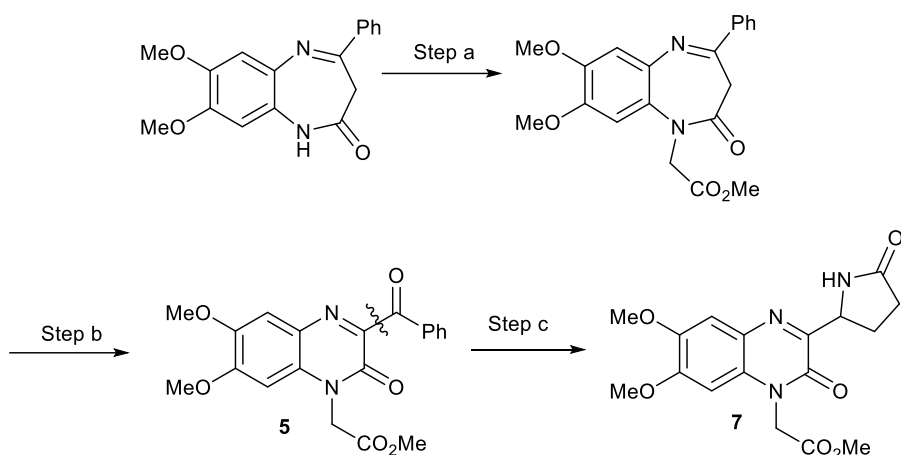
7,8-dimethoxy-1-methyl-4-phenyl-1,3-dihydro-2H-benzo[b][1,4]diazepin-2-one (Step b) To a solution of 7,8-dimethoxy-4-phenyl-1,3-dihydro-2H-benzo[b][1,4]diazepin-2-one (0.500 g, 1.7 mmol) in anhydrous THF (16 mL) at 0°C, was added NaH (60% in mineral oil, 82 mg, 3.41 mmol, 2 equiv). The mixture was stirred for 15 min before the addition of methyl iodide (0.212 mL, 3.41 mmol, 2 equiv). The reaction mixture was maintained at room temperature for 15 h. The solution was quenched with a saturated solution of NH₄Cl, extract with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel

(cyclohexane/EtOAc 50:50) and the desired product was obtained (0.360 g, 1.160 mmol, 68% yield) as yellow solid, mp = 203 °C. IR (neat): 3267, 2929, 1660, 1617, 1509, 1245, 1211, 1152, 1020, 605. ¹H NMR (300 MHz, CDCl₃): δ 8.14 – 8.06 (m, 2H), 7.52 – 7.44 (m, 3H), 6.93 (s, 1H), 6.75 (s, 1H), 4.16 (d, *J* = 12.2 Hz, 1H), 3.94 (s, 6H), 3.38 (s, 3H), 3.01 (d, *J* = 11.9 Hz, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 165.8, 159.2, 147.5, 146.6, 137.6, 135.6, 130.9, 128.8, 128.4, 127.6, 109.2, 104.4, 56.3, 56.2, 39.9, 35.4. HRMS (ESI+) Calculated for C₁₈H₁₉N₂O₃ [M+H]⁺: 311.1396; found: 311.1401.

3-benzoyl-6,7-dimethoxy-1-methylquinoxalin-2(1H)-one (3) (Step c). To a solution of 7,8-dimethoxy-1-methyl-4-phenyl-1,3-dihydro-2*H*-benzo[*b*][1,4]diazepin-2-one (0.268 g, 0.860 mmol) in DMSO (2.6 mL) in a flask equipped with a rubber septum (with a needle inserted for preventing overpressure), was added *N*-bromosuccinimide (61 mg, 0.344 mmol, 0.4 eq.) at room temperature. The mixture was then heated to 110 °C, and stirred for 6 h. Thereafter, to the solution cooled to room temperature were successively added dichloromethane (15 mL), a solution of saturated aq. NaHCO₃ (6 mL), and a solution of saturated aq. Na₂S₂O₃ (6 mL). The resulting organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude material was purified by flash column chromatography on silica gel (Cyclohexane/EtOAc from 100:0 to 50:50) to obtain **3** (202 mg, 0.623 mmol, 73% yield) as a yellow solid, mp = 215 °C. IR (neat): 3604, 3064, 2927, 1677, 1638, 1507, 1387, 1269, 1230, 1156, 1015. ¹H NMR (300 MHz, CDCl₃): δ 7.99 (m, 2H), 7.66 – 7.57 (m, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.35 (s, 1H), 6.75 (s, 1H), 4.07 (s, 3H), 3.95 (s, 3H), 3.76 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 192.1, 153.59, 153.5, 151.0, 146.8, 135.4, 134.0, 130.1, 129.9, 128.7, 126.8, 111.4, 95.9, 56.6, 56.4, 29.4. HRMS (ESI+) Calculated for C₁₈H₁₇N₂O₄ [M+H]⁺: 325.1188; found: 325.1185.

3-(hydroxy(phenyl)methyl)-6,7-dimethoxy-1-methylquinoxalin-2(1H)-one (4) (Step d). To a solution of **3** (20 mg, 0.062 mmol) in 3.2 mL of MeOH/THF 1:1 at 0 °C was added NaBH₄ (2.8 mg, 0.074 mmol, 1.2 equiv). The mixture was stirred for 10 min before the addition of water (15 mL). The crude was extracted with dichloromethane (3 x15 mL). The combined organic layers were dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (cyclohexane/EtOAc from 70:30 to 60:40) and the desired product **4** was obtained in quantitative yield (20 mg, 0.062 mmol) as a white solid, mp = 188 °C. IR (neat): δ 155.2, 153.8, 152.2, 146.6, 141.1, 128.7, 128.5, 127.9, 127.2, 126.4, 110.8, 96.0, 73.4, 56.6, 56.5, 29.32. ¹H NMR (300 MHz, CDCl₃): δ 7.58 – 7.50 (m, 2H), 7.38 (s, 1H), 7.32 (dd, *J* = 8.0, 6.4 Hz, 2H), 7.26 (m, 1H), 6.69 (s, 1H), 6.01 (d, *J* = 7.5 Hz, 1H), 5.06 (d, *J* = 7.5 Hz, 1H), 4.02 (s, 3H), 4.00 (s, 3H), 3.65 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 155.2, 153.8, 152.2, 146.6, 141.1, 128.7, 128.5, 127.9, 127.2, 126.4, 110.8, 96.0, 73.4, 56.6, 56.5, 29.32. HRMS (ESI+) Calculated for C₁₈H₁₉N₂O₄ [M+H]⁺: 327.1345; found: 327.1336.

Preparation of quinoxalinones 5 and 7

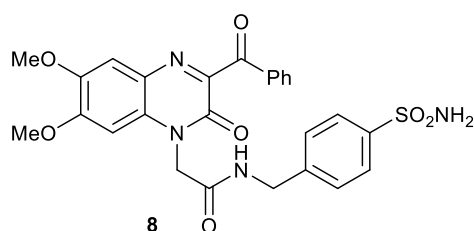


Methyl 2-(7,8-dimethoxy-2-oxo-4-phenyl-2,3-dihydro-1H-benzo[*b*][1,4]diazepin-1-yl)acetate (Step a). To a solution of 7,8-dimethoxy-4-phenyl-1,3-dihydro-2H-benzo[*b*][1,4]diazepin-2-one (0.200 g, 0.675 mmol) in anhydrous THF (3 mL) at 0 °C, was added NaH (60% in mineral oil, 33 mg, 1.35 mmol, 2 equiv). The mixture was stirred for 15 min before the addition of methyl 2-bromoacetate (0.26 mL, 2.7 mmol, 4 equiv). The reaction mixture was maintained at room temperature for 15 h. The solution was quenched with a saturated solution of NH₄Cl, extract with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (cyclohexane/EtOAc 50:50) and the desired product was obtained (0.231 g, 0.683 mmol, 93% yield) as a yellow solid, mp = 194 °C. IR (neat): 2998, 2960, 2921, 2855, 1752, 1674, 1511, 1442, 1257, 1200, 1021, 767. ¹H NMR (300 MHz, CDCl₃): δ 8.14 – 8.05 (m, 2H), 7.51 – 7.43 (m, 3H), 6.94 (s, 1H), 6.76 (s, 1H), 4.62 (d, *J* = 17.2 Hz, 1H), 4.21 (dd, *J* = 20.6, 14.6 Hz, 2H), 3.94 (s, 3H), 3.89 (s, 3H), 3.77 (s, 3H), 3.14 (d, *J* = 11.9 Hz, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 169.8, 165.8, 159.3, 147.8, 147.2, 137.7, 136.0, 131.1, 128.8, 127.7, 127.3, 109.2, 104.7, 56.4, 56.2, 52.7, 50.7, 39.8. HRMS (ESI+) Calculated for C₂₀H₂₁N₂O₅ [M+H]⁺: 369.1450; found: 369.1452.

Methyl 2-(3-benzoyl-6,7-dimethoxy-2-oxoquinoxalin-1(2H)-yl)acetate (5) (Step b). To a solution of methyl 2-(7,8-dimethoxy-2-oxo-4-phenyl-2,3-dihydro-1H-benzo[*b*][1,4]diazepin-1-yl)acetate (250 mg, 0.679 mmol) in DMSO (2 mL) in a flask equipped with a rubber septum (with a needle inserted for preventing overpressure), was added *N*-bromosuccinimide (48 mg, 0.272 mmol, 0.4 equiv) at room temperature. The mixture was then heated to 110 °C, and stirred for 12 h. Thereafter, to the solution cooled to room temperature were successively added dichloromethane (15 mL), a solution of saturated aq. NaHCO₃ (6 mL), and a solution of saturated aq. Na₂S₂O₃ (6 mL). The resulting organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 x 15 mL). The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude material was purified by flash column chromatography on silica gel (Cyclohexane/EtOAc from 100:0 to 50:50) to obtain **5** (215 mg, 0.562 mmol, 83% yield) as a yellow solid, mp = 56 °C. IR (neat): 3009, 2916, 2850, 1747, 1679, 1644, 1510, 1396, 1268, 1235, 1142. ¹H NMR (300 MHz, CDCl₃): δ 8.05 – 7.95 (m, 2H), 7.62 (m, 1H), 7.53 – 7.43 (m, 2H), 7.37 (s, 1H), 6.52 (s, 1H), 5.09 (s, 2H), 4.01 (s, 3H), 3.95 (s, 3H), 3.80 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 191.5, 167.3, 153.6, 152.8,

150.1, 146.7, 135.1, 133.9, 129.8, 128.8, 128.4, 126.6, 111.3, 95.5, 56.4, 56.1, 52.8, 43.5. HRMS (ESI+) Calculated for C₂₀H₁₉N₂O₆ [M+H]⁺: 383.1243; found: 283.1248.

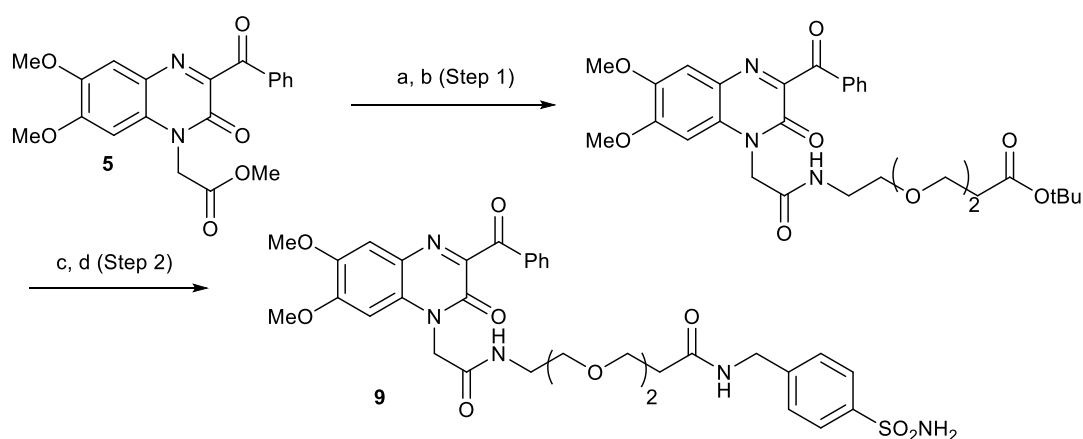
Methyl 2-(6,7-dimethoxy-2-oxo-3-(5-oxopyrrolidin-2-yl)quinoxalin-1(2H)-yl)acetate (7) (Step c). Quinoxalinone **5** (30 mg, 0.078 mmol) in 2-pyrrolidinone (3.360 g, 39.5 mmol, 506 equiv) was irradiated in a glass tube with a blue LED lamp at 450 nm for 63 h. The reaction was monitored by analytical RP-HPLC. When conversion reached 31%, the crude was directly purified by semi-preparative RP-HPLC to obtain **7** (3.1 mg, 0.009 mmol, 11% yield) as a yellow oil. IR (neat): 1764, 1679, 1648, 1514, 1398, 1243. ¹H NMR (300 MHz, CDCl₃): δ 7.33 (s, 1H), 6.48 (s, 1H), 6.28 (s, 1H), 5.15 – 5.06 (m, 1H), 5.04 (s, 2H), 3.96 (s, 6H), 3.79 (s, 3H), 2.75 – 2.57 (m, 1H), 2.56 – 2.38 (m, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 178.3, 167.6, 153.9, 153.8, 152.5, 146.9, 127.6, 126.9, 111.6, 95.5, 56.6, 56.5, 55.4, 53.2, 43.8, 29.8, 24.9.



2-(3-benzoyl-6,7-dimethoxy-2-oxoquinoxalin-1(2H)-yl)-N-(4-sulfamoylbenzyl)acetamide (8). To a solution of **5** (52 mg, 0.136 mmol) in THF (2.7 mL) was added a solution of LiOH (13 mg, 0.544 mmol, 4 equiv) in 0.3 mL of water. The mixture was stirred for 5 h at 50°C. The solution was quenched for 10 min with a solution of hydrochloric acid (0.5 mL (2 N)) before the addition of water (5 mL). The crude was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄ and evaporated under vacuum. The crude material was directly engaged in the next step without purification.

To a solution of the previous carboxylic acid in anhydrous DCM (570 μL) and anhydrous DMF (130 μL) was added EDCI (26 mg, 0.163 mmol, 1.2 equiv) and the mixture was stirred for 15 min before the addition of 4-(aminomethyl)benzenesulfonamide (30 mg, 0.163 mmol, 1.2 equiv). The mixture was stirred for 3 h at room temperature. The solution was quenched with a saturated solution of NH₄Cl (15 mL), extract with EtOAc (3 x 15 mL). The combined organic layers were washed with brine, dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (DCM/MeOH 95:5) to obtain **8** (23 mg, 0.043 mmol, 31% yield) as a yellow solid, mp = 197 °C. IR (neat): 3272, 3069, 2937, 1664, 1512, 1402, 1234, 1157. ¹H NMR (300 MHz, DMSO): δ 8.88 (t, *J* = 5.9 Hz, 1H), 7.97 – 7.88 (m, 2H), 7.72 (dd, *J* = 9.6, 4.8 Hz, 3H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.49 – 7.40 (m, 3H), 7.32 (s, 2H), 6.97 (s, 1H), 5.09 (s, 2H), 4.40 (d, *J* = 5.8 Hz, 2H), 3.85 (d, *J* = 4.4 Hz, 6H). ¹³C NMR (75 MHz, DMSO): δ 192.2, 166.1, 153.1, 152.8, 150.5, 146.3, 143.2, 142.8, 135.0, 134.4, 129.6, 129.5, 128.9, 127.5, 126.3, 125.6, 110.8, 97.2, 56.3, 56.0, 45.1, 41.9. HRMS (ESI+) Calculated for C₂₆H₂₅N₄O₇S [M+H]⁺: 537.1458; found: 537.1444.

3-(2-(2-(2-(3-benzoyl-6,7-dimethoxy-2-oxoquinoxalin-1(2H)-yl)acetamido)ethoxy)ethoxy)-N-(4-sulfamoylbenzyl)propanamide (9).



Step 1: tert-butyl 3-(2-(2-(2-(3-benzoyl-6,7-dimethoxy-2-oxoquinoxalin-1(2H)-yl)acetamido)ethoxy)ethoxy)propanoate. To a solution of **5** (22 mg, 0.058 mmol) in THF (1.1 mL) was added a solution of LiOH (6 mg, 0.232 mmol, 4 equiv) in 0.1 mL of water. The mixture was stirred for 5 h at 50°C. The solution was quenched for 10 min with a solution of hydrochloric acid (0.2 mL (2 N)) before the addition of water (2 mL). The crude was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO₄ and evaporated under vacuum. The crude material was directly engaged in the next step without purification.

To a solution of the previous carboxylic acid in anhydrous DCM (240 μL) and anhydrous DMF (60 μL) was added EDCI (13 mg, 0.070 mmol, 1.2 equiv) and the mixture was stirred for 15 min before the addition of tert-butyl 3-(2-(2-aminoethoxy)ethoxy)propanoate (16 mg, 0.070 mmol, 1.2 equiv). The mixture was stirred for 3 h at room temperature. The solution was quenched with a saturated solution of NH₄Cl (5 mL), extract with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (DCM/MeOH 95:5) to obtain the compound (8 mg, 0.014 mmol, 35% yield) as a yellow solid, mp = 111 °C. IR (neat): 3321, 2926, 1723, 1669, 1621, 1539, 1511, 1453, 1406, 1271, 1234, 1121, 847, 730. ¹H NMR (300 MHz, CDCl₃): δ 8.01 – 7.94 (m, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.47 (t, J = 7.6 Hz, 2H), 7.31 (s, 1H), 7.09 – 7.05 (m, 1H), 7.04 (s, 3H), 4.95 (s, 2H), 4.04 (s, 3H), 3.92 (s, 3H), 3.64 (t, J = 6.3 Hz, 2H), 3.50 (s, 6H), 3.47 – 3.32 (m, 2H), 2.45 (t, J = 6.3 Hz, 2H), 1.41 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ 191.6, 171.3, 166.4, 154.0, 153.6, 150.1, 147.2, 135.4, 134.1, 130.3, 129.5, 128.7, 127.1, 111.2, 96.9, 80.7, 70.39, 70.37, 69.5, 66.9, 56.8, 56.4, 47.1, 39.7, 36.4, 28.2. HRMS (ESI+) Calculated for C₃₀H₃₈N₃O₉ [M+H]⁺: 584.2608; found: 584.2615.

Step 2: 3-(2-(2-(2-(3-benzoyl-6,7-dimethoxy-2-oxoquinoxalin-1(2H)-yl)acetamido)ethoxy)ethoxy)-N-(4-sulfamoylbenzyl)propanamide **9**. To a solution of the previous ester (89 mg, 0.152 mmol) in anhydrous DCM (1.5 mL) at 0°C was added TFA (17%, 300 μL). The mixture was stirred for 1 h 30 before evaporated under vacuum to give the carboxylic acid without further purification.

To a solution of the previous carboxylic acid in anhydrous DCM (630 μL) and anhydrous DMF (150 μL) was added EDCI (35 mg, 0.182 mmol, 1.2 equiv) and the mixture was stirred for

15 min before the addition of 4-(aminomethyl)benzenesulfonamide (34 mg, 0.182 mmol, 1.2 equiv). The mixture was stirred for 3 h at room temperature. The solution was quenched with a saturated solution of NH₄Cl (15 mL), extract with EtOAc (3 x 15 mL). The combined organic layers were washed with brine, dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (DCM/MeOH 95:5) to obtain **9** (16 mg, 0.229 mmol, 15% yield) as a yellow solid, mp = 85 °C. IR (neat): 3263, 3075, 2927, 1663, 1512, 1403, 1329, 1234, 1155, 995. ¹H NMR (300 MHz, CDCl₃): δ 7.95 (d, J = 7.2 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 7.63 (s, 1H), 7.48 (t, J = 7.5 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.30 (s, 1H), 7.22 – 7.13 (m, 2H), 7.00 (s, 1H), 5.46 (s, 2H), 4.74 (s, 2H), 4.46 (d, J = 5.6 Hz, 2H), 4.04 (s, 3H), 3.93 (s, 3H), 3.76 – 3.68 (m, 2H), 3.56 – 3.47 (m, 4H), 3.41 – 3.34 (m, 2H), 3.39 – 3.29 (m, 2H), 2.53 (t, J = 5.2 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 192.0, 172.2, 166.7, 154.6, 153.7, 149.0, 147.5, 144.1, 141.2, 135.3, 134.4, 130.48, 129.5, 128.8, 128.0, 127.2, 126.6, 111.2, 96.8, 70.2, 69.2, 67.4, 56.9, 56.5, 47.0, 42.9, 39.4, 37.1, 29.8. HRMS (ESI+) Calculated for C₃₃H₃₇N₅O₁₀SNa [M+H]⁺: 718.2159; found: 718.2147.

N-(4-sulfamoylbenzyl)acetamide (10). To a solution of 4-(aminomethyl)benzenesulfonamide (25 mg, 0.134 mmol) in anhydrous DMF (800 μL) at 0 °C was added acetic anhydride (27 μL, 0.282 mmol, 2.1 equiv). The mixture was stirred 1.5 h before evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (EtOAc/MeOH 95:5) to obtain **10** (22 mg, 0.096 mmol, 72% yield) as a white solid. ¹H NMR (300 MHz, MeOH-d₄) δ 7.86 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 4.43 (s, 2H), 2.01 (s, 3H). ¹³C NMR (75 MHz, MeOH-d₄) δ 173.3, 144.7, 143.8, 128.9, 127.4, 43.6, 22.5.

3-(((2-(2-(3-benzoyl-6,7-dimethoxy-2-oxoquinoxalin-1(2H)-yl)acetamido)ethoxy)(hydroxy)phosphoryl)oxy)propane-1,2-diyl dioleate (11). To a solution of **5** (11 mg, 0.028 mmol) in THF (0.6 mL) was added a solution of LiOH (3 mg, 0.112 mmol, 4 equiv) in 0.1 mL of water. The mixture was stirred for 5 h at 50 °C. The solution was quenched for 10 min with a solution of hydrochloric acid (0.5 mL (2 N)) before the addition of water (1 mL). The crude was extracted with EtOAc (3 x 4 mL). The combined organic layers were dried over MgSO₄ and evaporated under vacuum. The crude material was directly engaged in the next step without purification.

To a solution of the previous carboxylic acid in anhydrous DCM (120 μL) and anhydrous DMF (30 μL) was added EDCI (6 mg, 0.034 mmol, 1.2 equiv) and the mixture was stirred for 15 min before the addition of 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (25 mg, 0.034 mmol, 1.2 equiv). The mixture was stirred overnight at room temperature. The solution was quenched with a saturated solution of NH₄Cl (1 mL), extract with EtOAc (3 x 1 mL). The combined organic layers were washed with brine, dried over MgSO₄ and evaporated under vacuum. The crude material was finally purified by flash column chromatography on silica gel (DCM/MeOH 95:5) to obtain **11** (12 mg, 0.011 mmol, 39% yield) as a yellow solid. ¹H NMR (300 MHz, CDCl₃): δ 8.73 (s, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.50 (t, J = 7.8 Hz, 1H), 7.35 (t, J = 7.7 Hz, 2H), 7.20 (s, 1H), 7.00 (s, 1H), 5.33 (m, 4H), 5.09 (d, J = 18.8 Hz, 3H), 4.26 (d, J = 9.9 Hz, 2H), 4.03 (s, 4H), 3.91 (s, 6H), 3.41 (s, 3H), 2.20 (m, 8H), 1.97 (m, 10H), 1.49 (s, 12H), 1.25 (s, 72H), 0.87 (m, 14H). ¹³C NMR (75 MHz, CDCl₃) δ 191.8, 173.5, 173.3, 166.9, 155.0, 154.4, 147.5, 135.8, 133.7, 130.8, 130.5, 130.1, 129.8, 128.4, 127.1, 111.0, 97.0, 70.6, 57.0, 56.4, 34.1, 32.1,

32.0, 31.9, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.2, 27.4, 27.4, 25.0, 24.9, 22.8, 22.8, 14.3, 9.0. HRMS (ESI-) Calculated for C₆₀H₉₁N₃O₁₃P [M-H]⁻: 1092.6290; found: 1092.6294.

Labelling procedure of CA-II with quinoxalinones **3**, **8**, and **9**

Labelling of isolated CA-II: To a solution of CA-II (0.3 mg, 10 μM) in PBS (1 mL, pH 7.4, 0.1 M) was added the corresponding 3-benzoylquinoxalinone (**3**, **8** or **9**) in a 10 mM DMSO stock solution to reach the following final concentrations: CA-II (10 μM), 3-benzoylquinoxalinone (5, 9 or 11, 10 μM). Then, the mixture was incubated for 30 min at 0 °C. For the competition experiment, CA-II was pre-incubated with acetazolamide at 0 °C for 30 min before addition of compound **8**. The samples were irradiated in a glass tube with a blue LED lamp at 450 nm for 1 h, and air flow was used to conserve the sample at room temperature.

Labelling of CA-II mixed with HBP: To a solution of CA-II (3 mg/mL, 100 μM) in HBP (diluted 10 times in PBS pH 7.4, 0.1 M) was added compound **3** or **8** in a 10 mM DMSO stock solution, to reach the following final concentrations: CA-II (100 μM), compound **3** or **8** (100 μM).

Then, the mixture was incubated for 30 min at 0 °C. The samples were irradiated in a glass tube with a blue LED lamp at 450 nm for 2 h. Air flow was used to cool the samples.

Procedure for SDS-PAGE analysis

First, a Bradford assay was performed according to the manufacturer's instructions (Bio-Rad protein Assay Dye Reagent concentrate, Biorad) to estimate the protein concentration of (1) labelling of isolated CA-II and (2) labelling of CA-II mixed with HBP. For the two experiments, appropriate dilution was made to take 2 μg of CA-II and mix it with 2X Laemmli buffer (0.15 M Tris-NH₂, 4% SDS, 17% glycerol, 0.008% bromophenol blue and 0.1 M DTT). Samples were heated for 5 min at 95 °C and then centrifuged for 15 seconds at 2500g. Thereafter, proteins were separated by SDS-PAGE in a 12.5% polyacrylamide gel (acrylamide/bis-acrylamide 30% [29 : 1], Sigma) with 0.05% of SDS. Molecular masses were estimated using SigmaMarker™ wild range (Sigma). After migration (10 mA per gel) and gel fixation (50% H₂O, 40% EtOH and 10% acetic acid), proteins were revealed successively by fluorescence and by silver nitrate staining. First, the fluorescence was directly estimated using a ChemiDoc MP Imaging System (Biorad). The fluorescence images were obtained with a 302 nm laser and a 590 nm emission filter (± 110 nm). Second, the gel was stained with nitrate silver. Briefly, the gel was sensitized for 1 min with 0.02% sodium thiosulfate (Sigma). Then, the gel was stained for 20 min with 0.1% silver nitrate solution (Sigma). Finally, the gel was developed in a specific buffer (0.0037% formaldehyde, 1.2% sodium carbonate and 0.00008% sodium thiosulfate in distilled water). The development was stopped after sufficient staining with 5% acetic acid. Silver nitrate coloration was read using a conventional scanner (Image Scanner III, GE Healthcare).

Procedure for cell imaging

Cell Culture

As previously described,⁵ tumoral PC12 cells, derived from a rat adrenal medulla pheochromocytoma, were cultured in a humidified incubator at 37 °C with an atmosphere of 5 % CO₂. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Illkirch, France) supplemented with 7 % heat-inactivated fetal bovine serum (Sigma–

Aldrich), 7 % horse serum (Lonza Bioscience, Walkersville, MD, USA), 2.5 % HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (Thermo Fisher Scientific), 1 % glutamine (Thermo Fisher Scientific), 100 units/mL penicillin and 100 $\mu\text{g mL}^{-1}$ streptomycin (Thermo Fisher Scientific).

Cell labelling

For fluorescent labelling, PC12 cells were plated on glass bottom dishes (grade 1.5 MatTek Corporation, MA, USA). After 1 day of culture, cells were fixed with paraformaldehyde (PFA) 4% during 5 min at room temperature. Prior dilution in PBS, solubilization of functionalizable quinoxalinone **11**, which is equipped with a phospholipid, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, was facilitated with the nonionic surfactant polyol Pluronic® F-127 (ThermoFisher Scientific, Montigny le Bretonneux, France) (v/v). In PBS, cells were incubated for 1 h at room temperature with different concentrations of probe **11** (10^{-3} M, 10^{-4} M or 10^{-5} M with < 0.1% DMSO) or in absence of probe for control experiment. Then, dishes were placed in a 450 nm-LED strip-containing beaker and were homogeneously irradiated for 1 h. A similar set of dishes was not exposed to blue light as control experiments. After exposure, cells were directly mounted in PBS with an additional coverslip directly placed in MatTek culture dishes.

Confocal microscopy

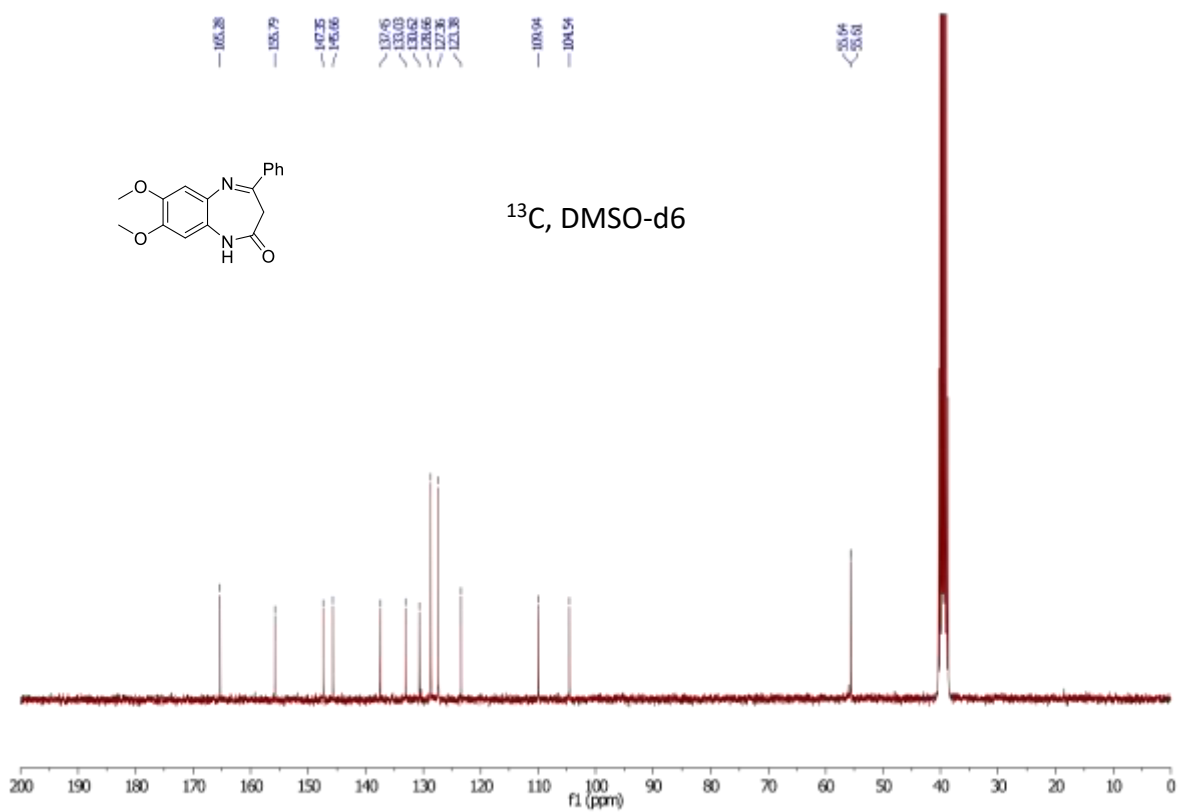
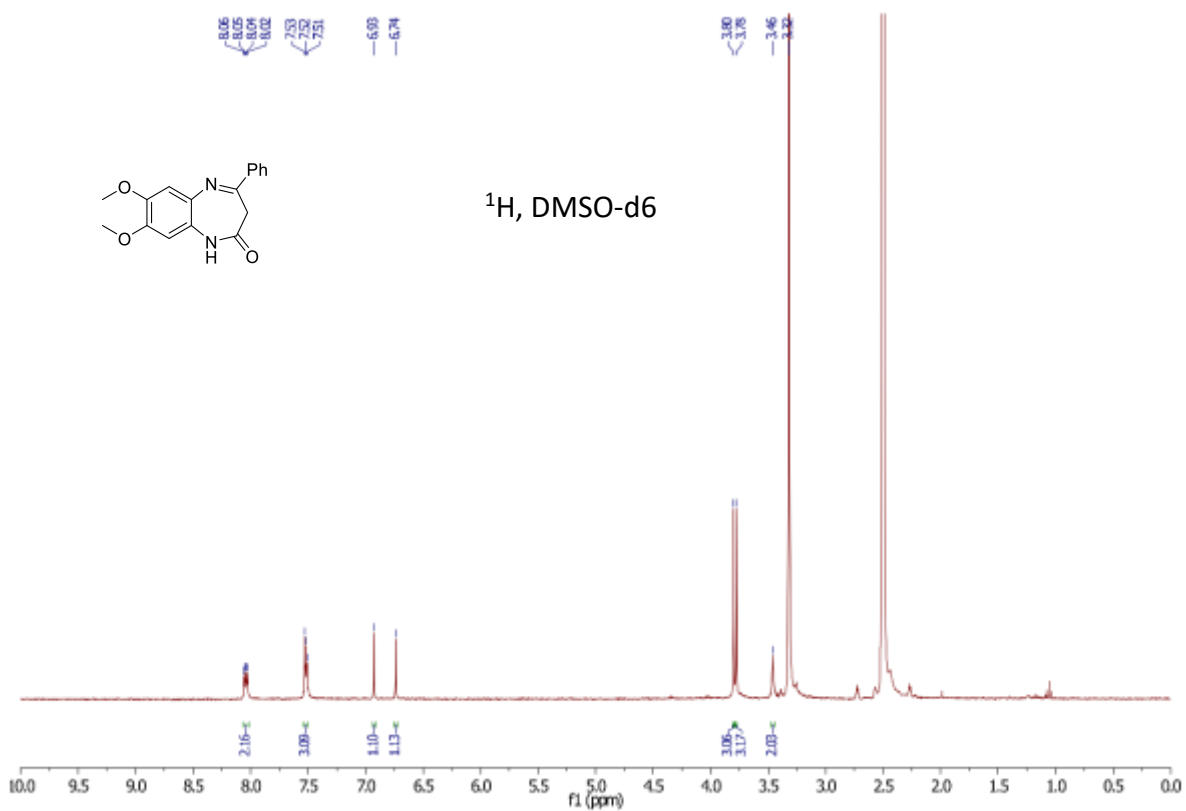
Cell imaging was performed on an upright fixed stage Leica TCS SP8 confocal microscope (Leica Microsystems, Nanterre, France) equipped with a 405 nm-diode laser source and conventional scanner (400 Hz). Using a 63x objective (N.A. = 1.4, oil immersion), fluorescence emission (450-475 nm) was detected through a hybrid detector (HyD) in photon counting mode. Through module LAS X Navigator, mosaics (5x5) of images (each Image Size = 175.91 x 175.91 μm at 1024 x 1024 pixels; optical zoom = 0.75; merged Image Size = 804.30 x 807.74 μm at 4682 x 4702 pixels, optical zoom = 0.75) were obtained to illustrate cell population labelling. Higher magnifications and Z-stacks acquisitions were used to describe subcellular details. For figure 5C and 5D: image Size = 87.95 x 87.95 μm at 1024 x 1024 pixels; optical zoom = 2.0; z-step = 300 nm; z-stack = 7.2 μm , $z_1=0.75$ μm , $z_2=1.8$ μm .

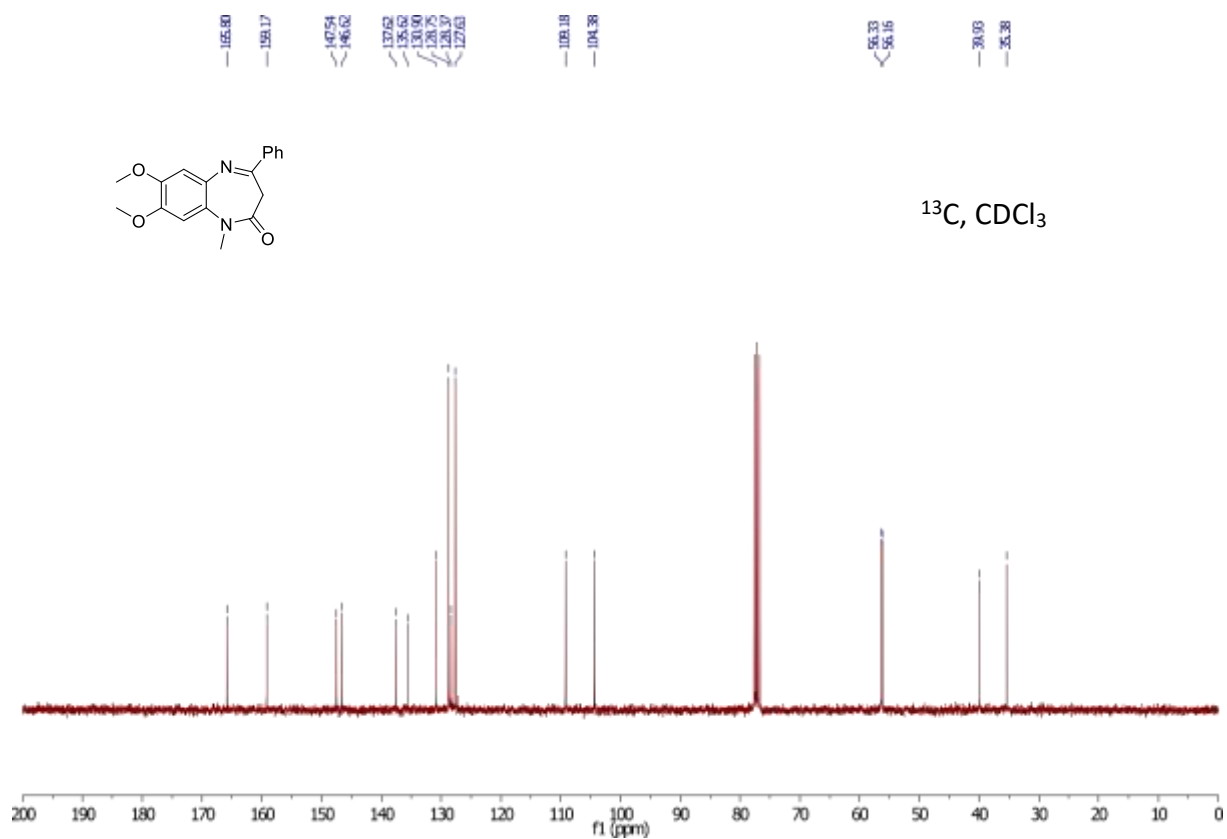
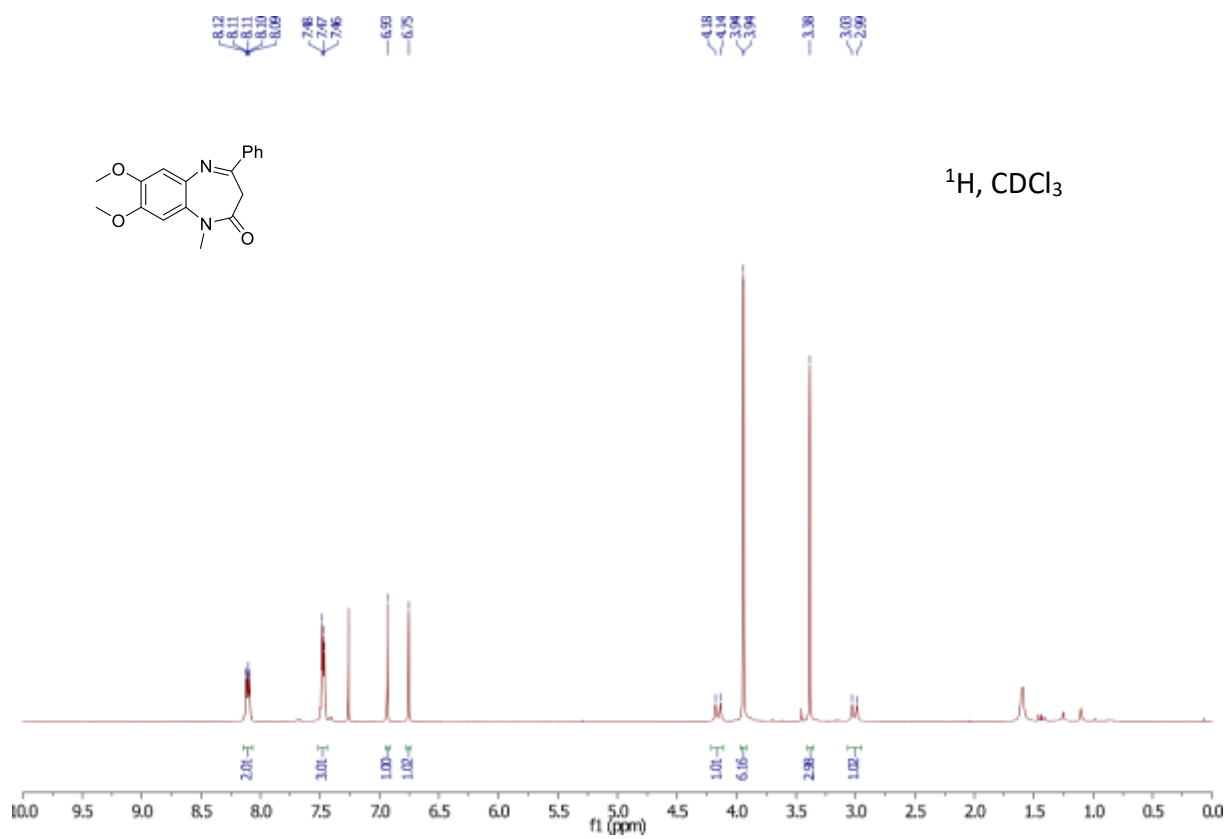
Image Analysis

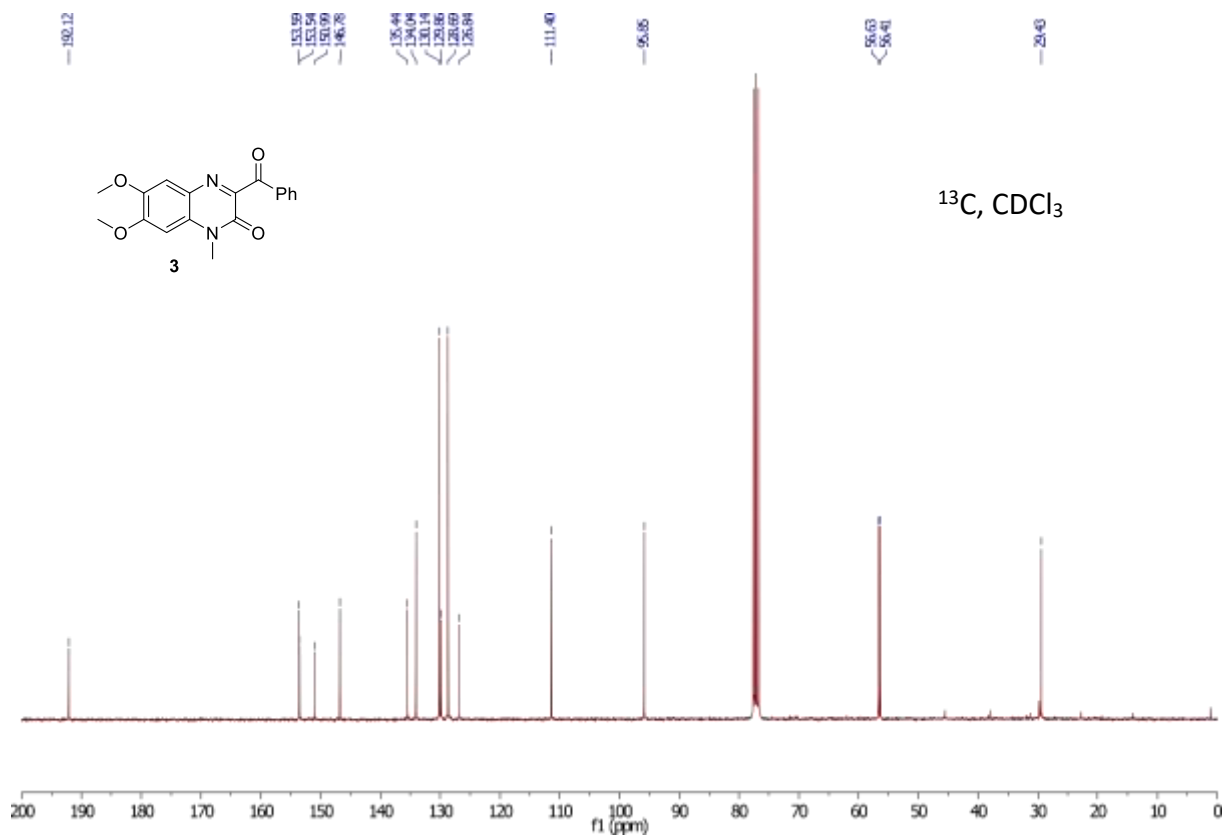
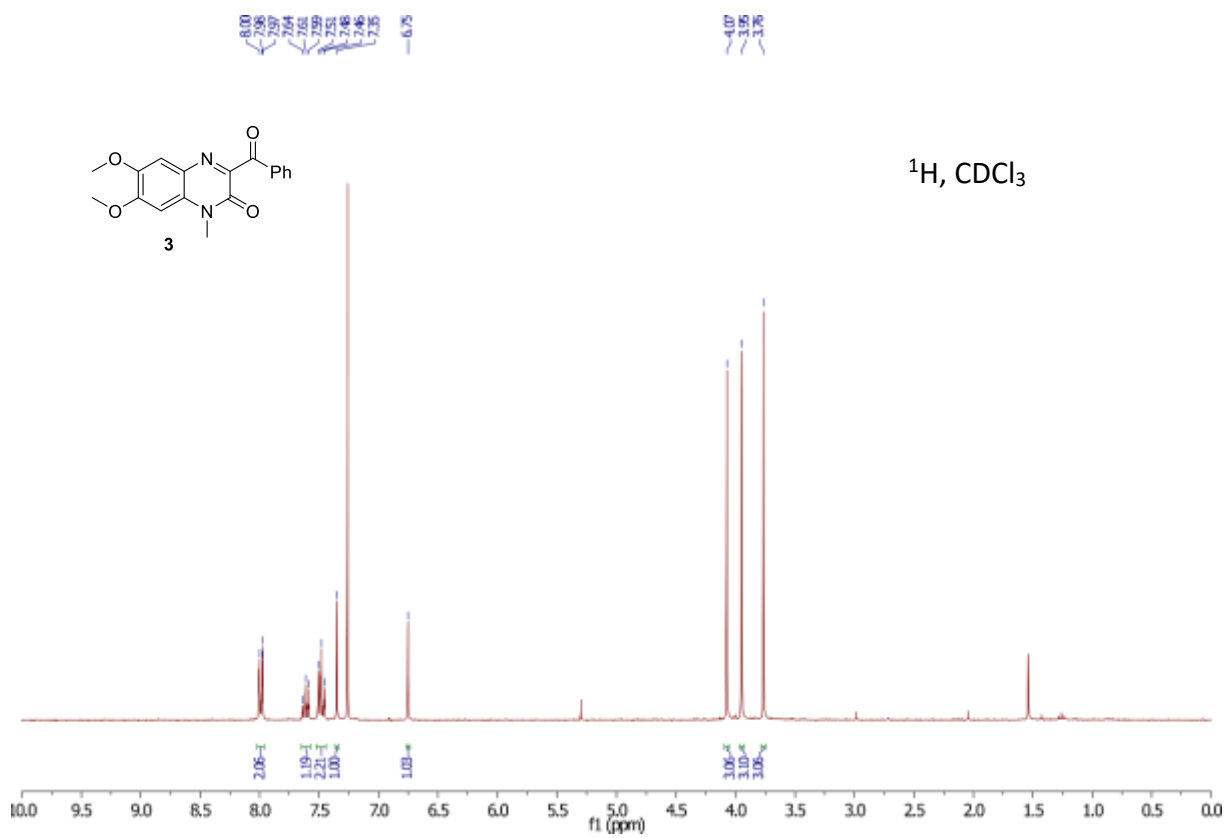
Fluorescence intensities of irradiated and non-irradiated cells incubated with different concentrations (10^{-3} M, 10^{-4} M or 10^{-5} M) of probe **11** were extracted from merged images and compared. Statistical analysis by using t-test with the Kolmogorov and Smirnov method was performed with the GraphPad Prism 4 software (GraphPad Software Inc., San Diego, CA, USA). Each value represents the mean (\pm S.E.M.) of fluorescence intensity of at least 60 cells detected in respective merged image. * $p<0.05$ for irradiated vs. non-irradiated cells previously incubated with probe **11** (10^{-5} M); *** $p<0.001$ for irradiated vs. non-irradiated cells previously incubated with probe **11** (10^{-4} M or 10^{-3} M).

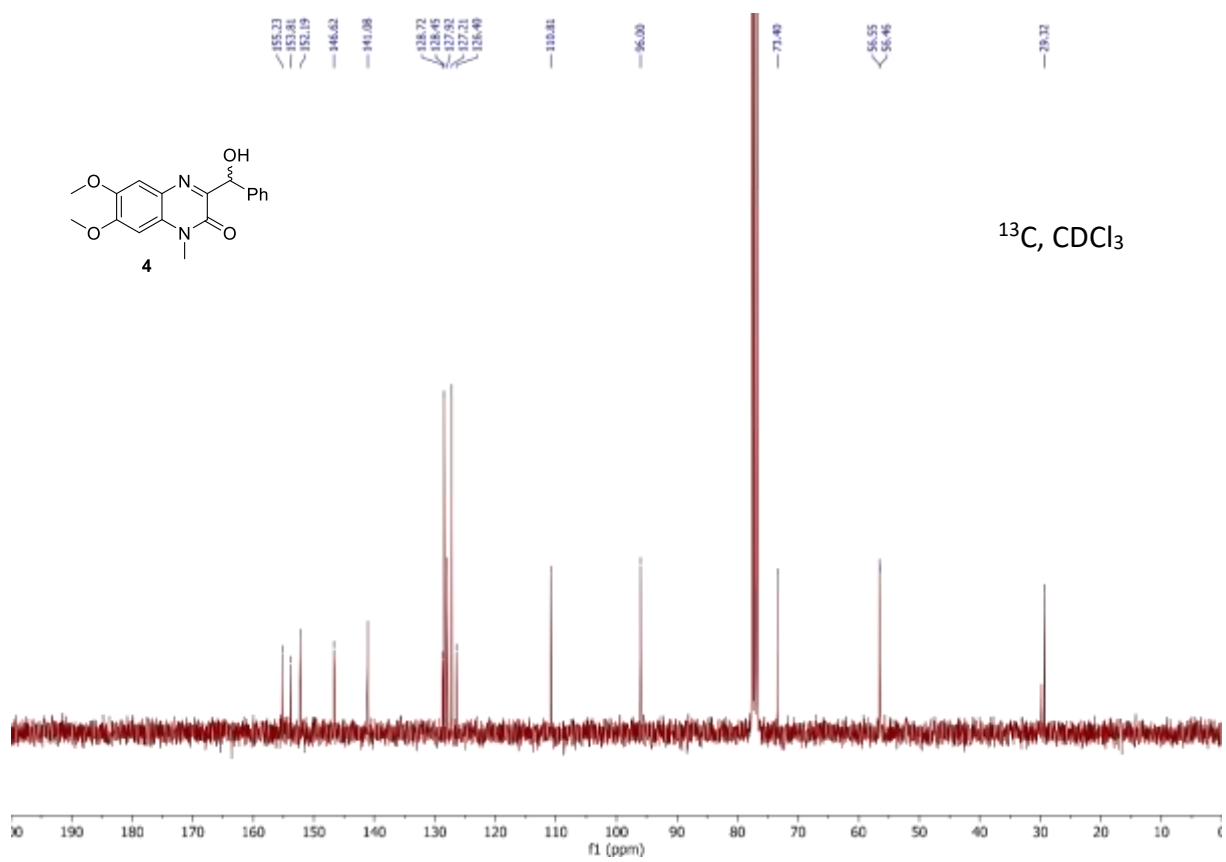
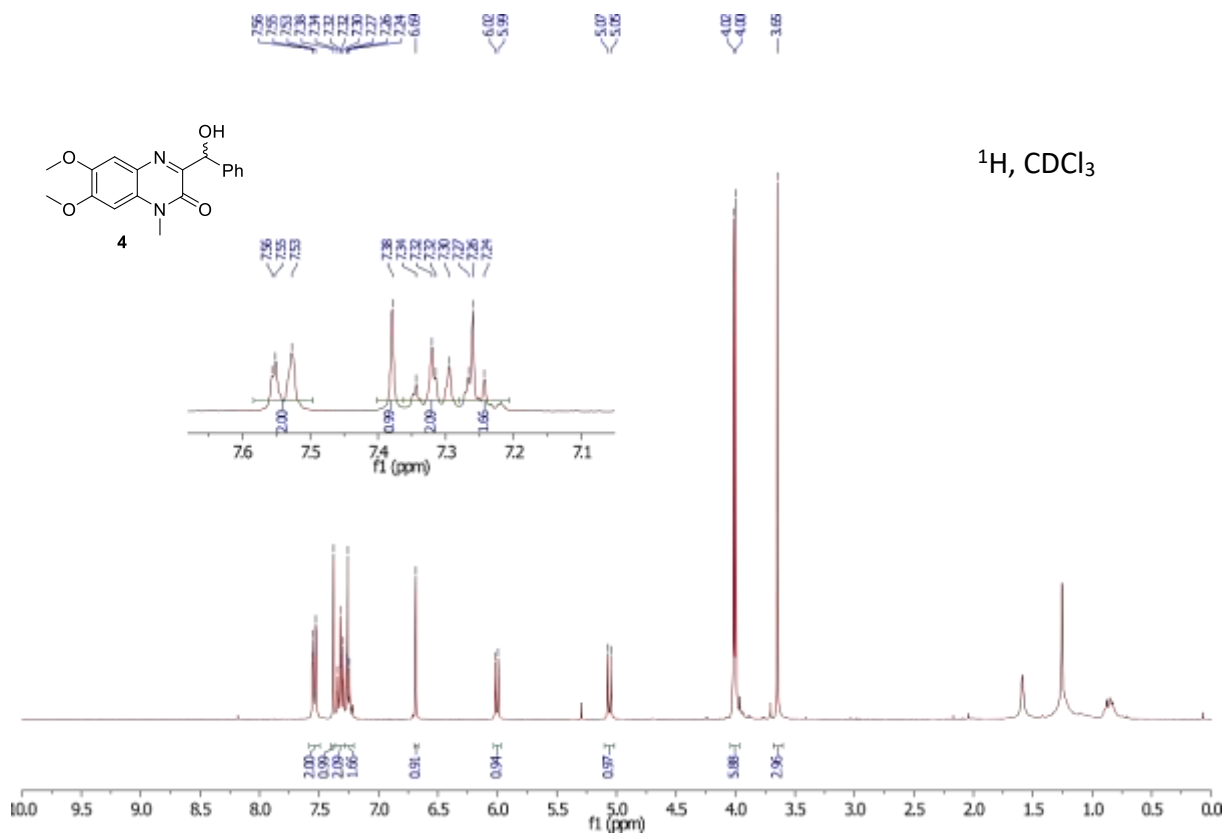
ImageJ (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2016) was used to adjust image brightness and contrast and to perform projections of 3D images (xyz).

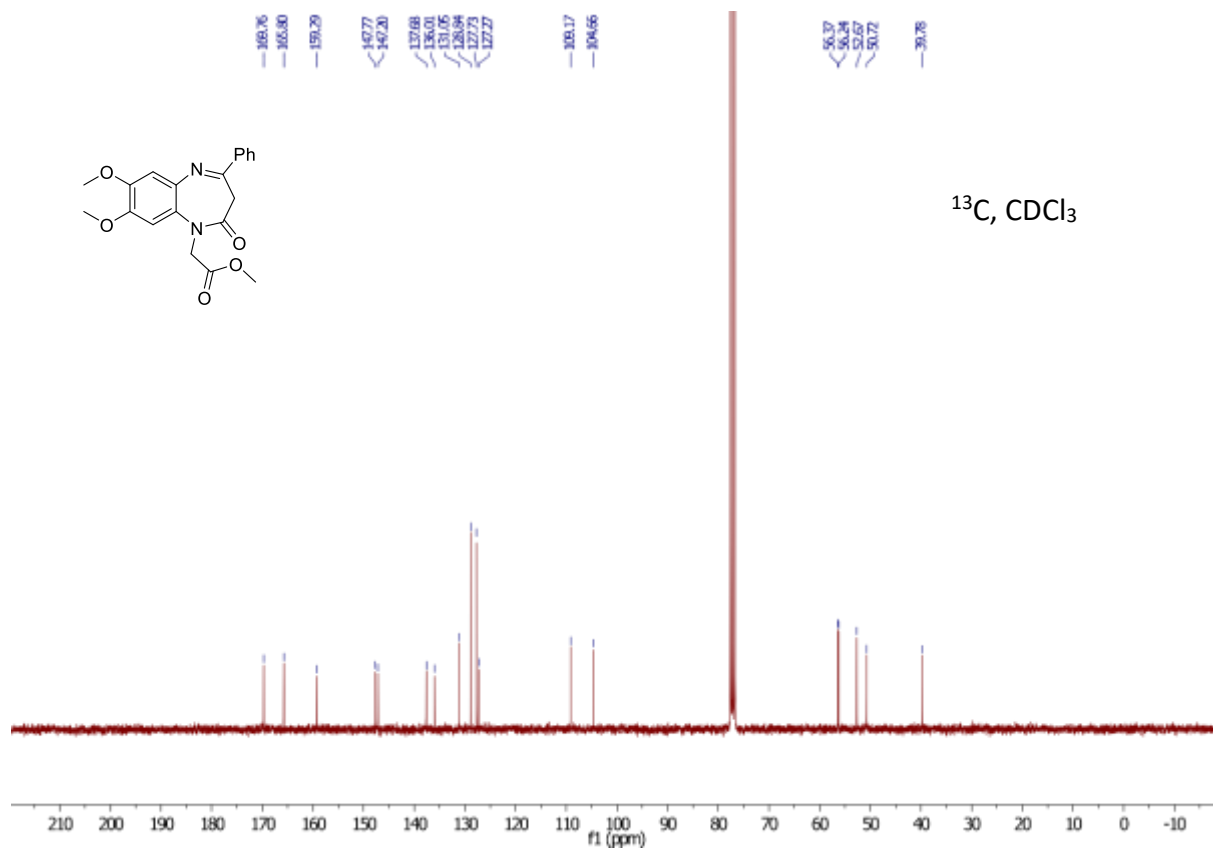
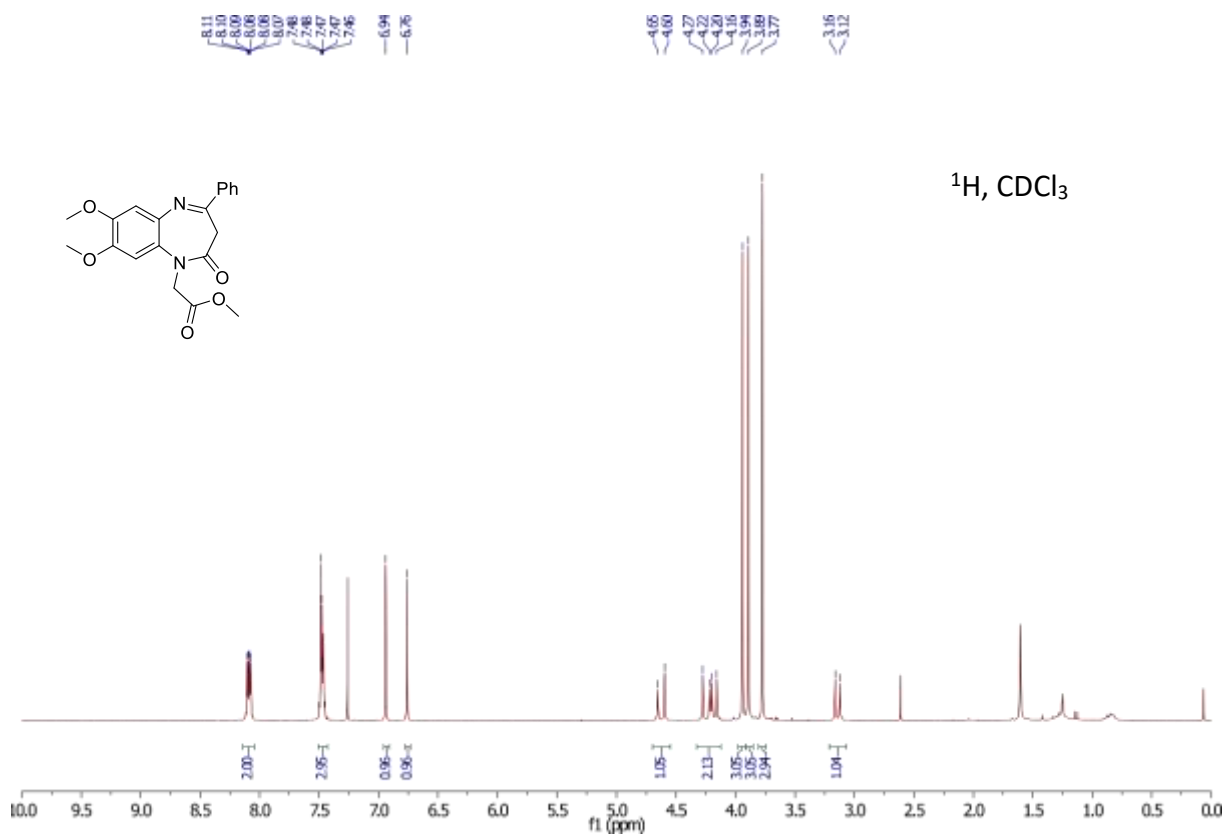
III. Copies of ^1H and ^{13}C NMR spectra

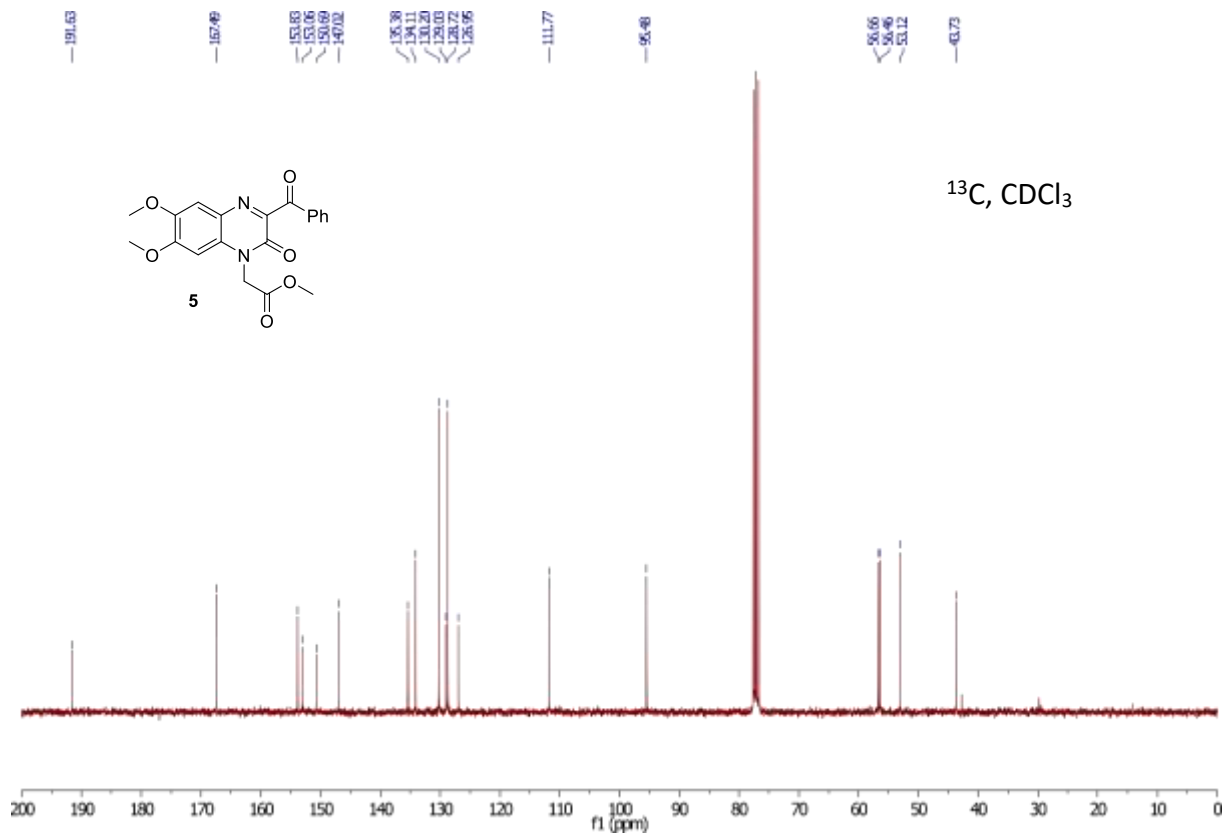
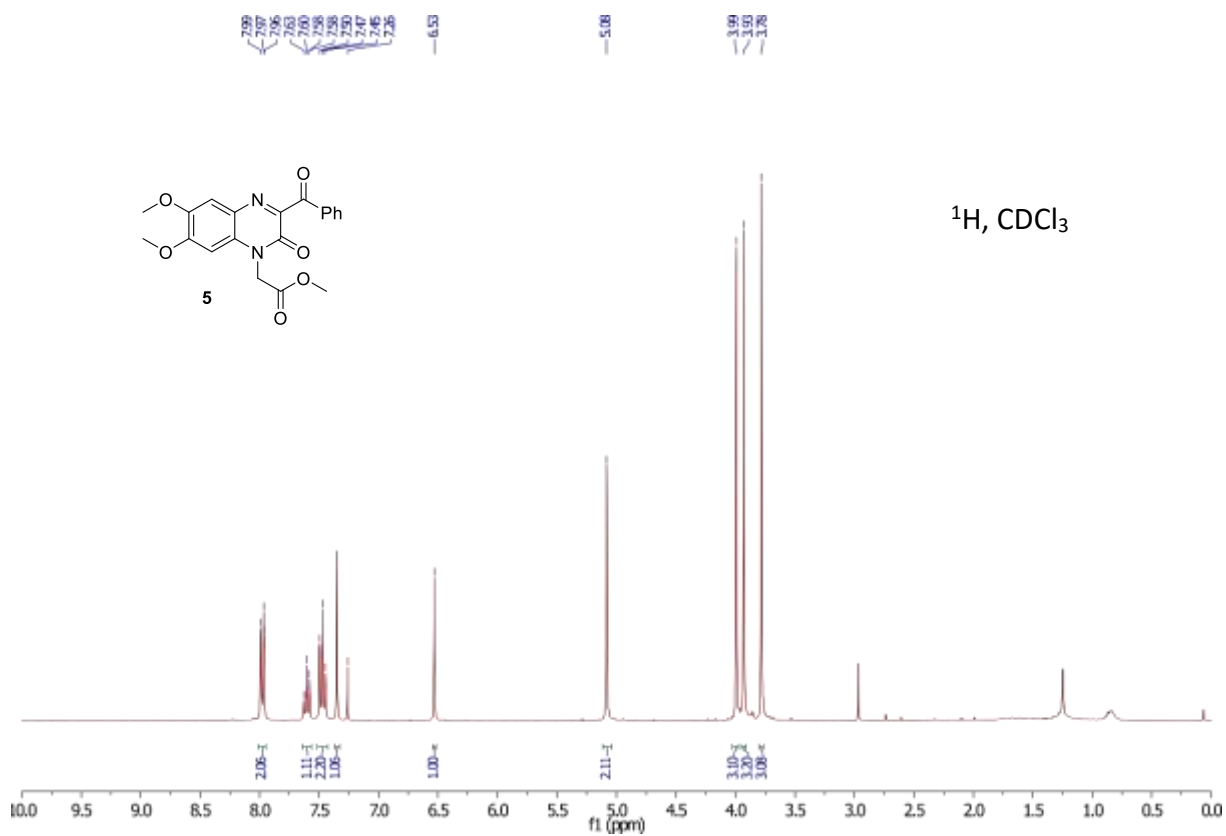


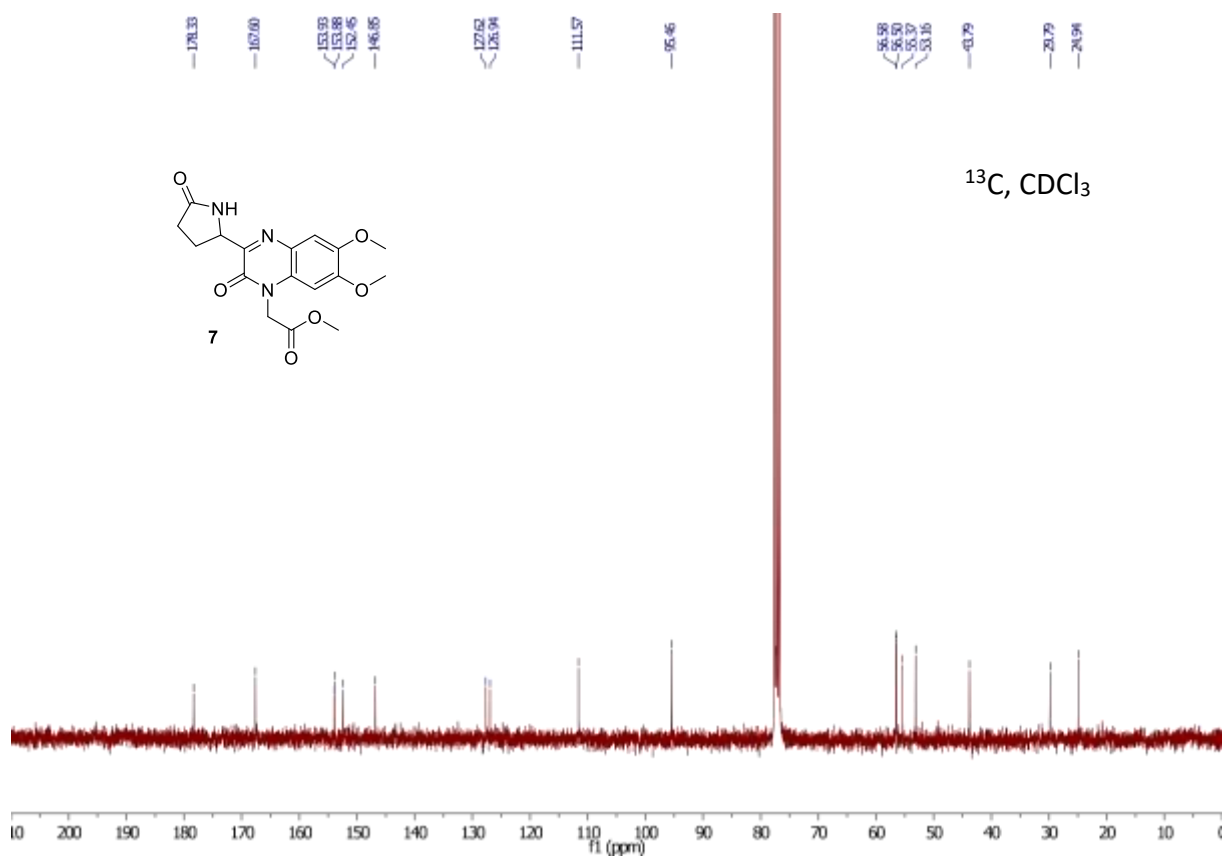
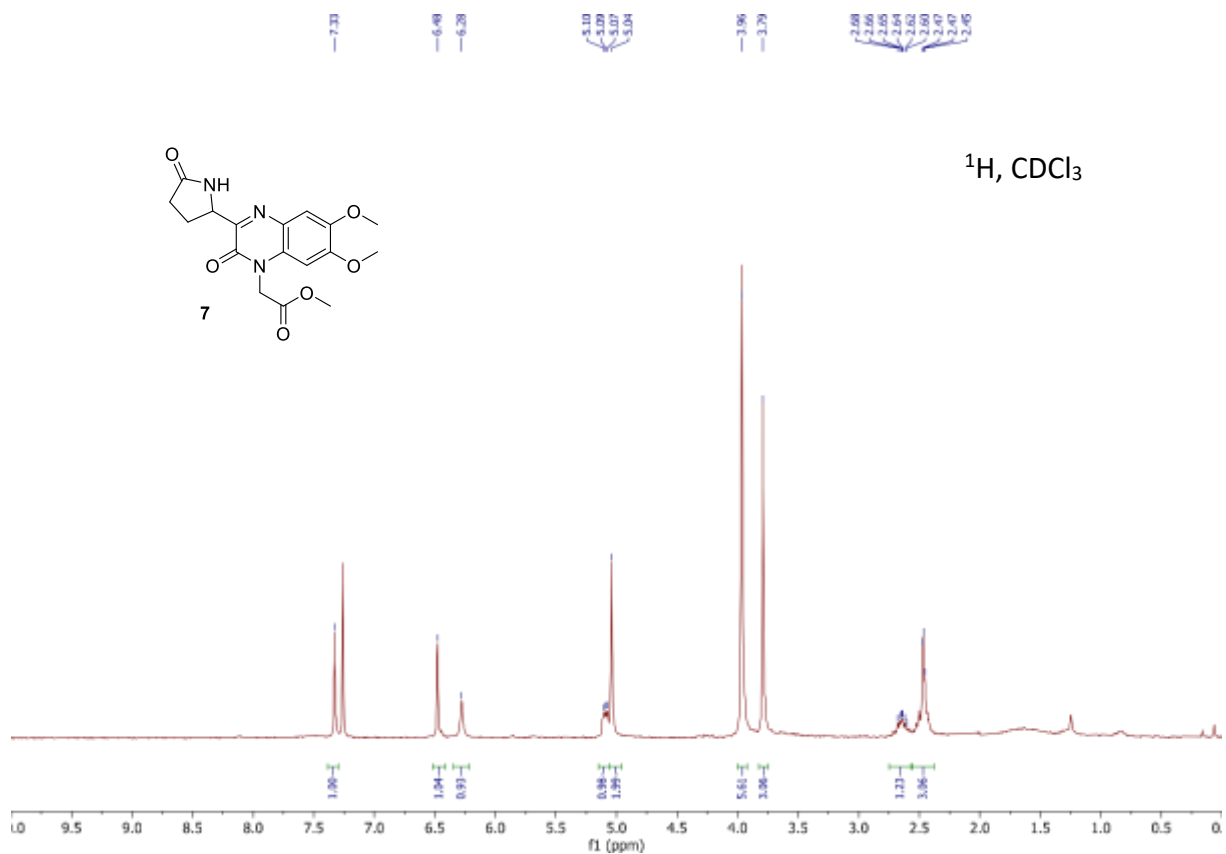


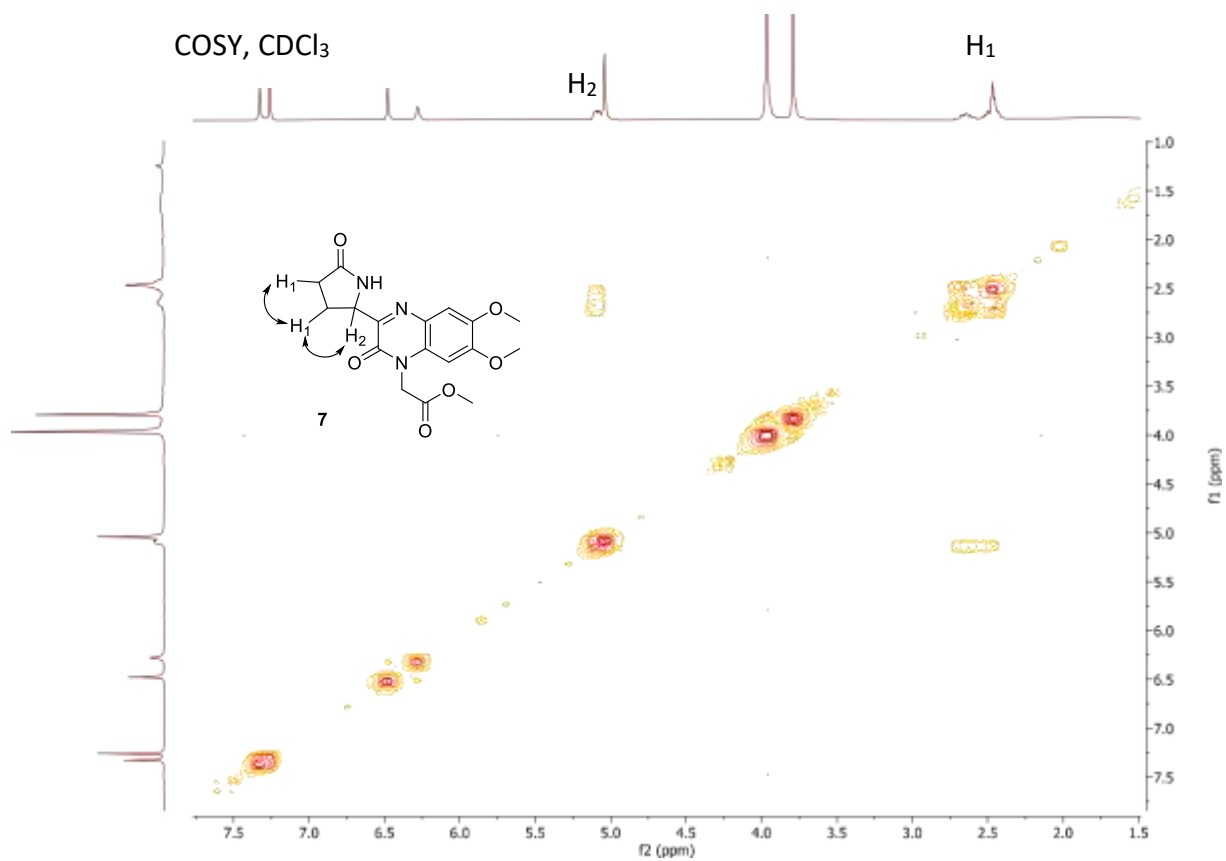


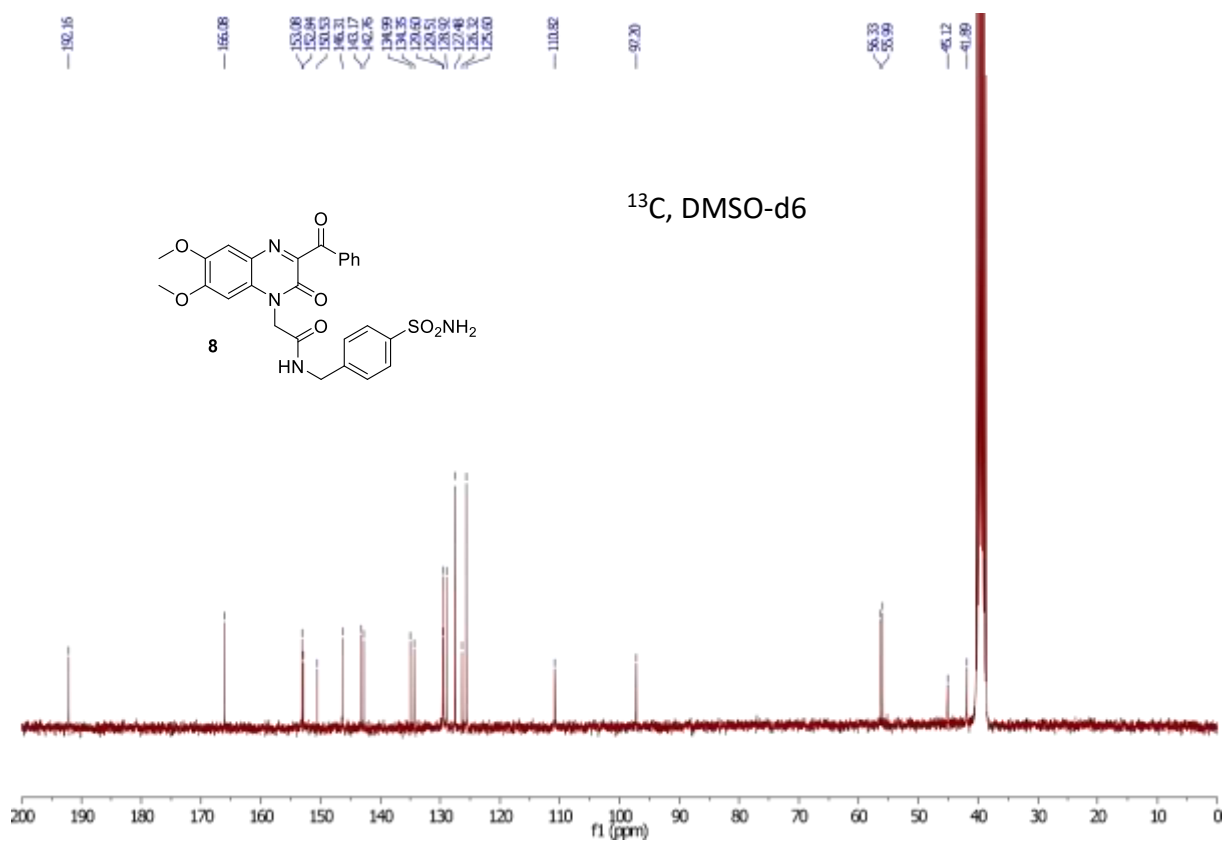
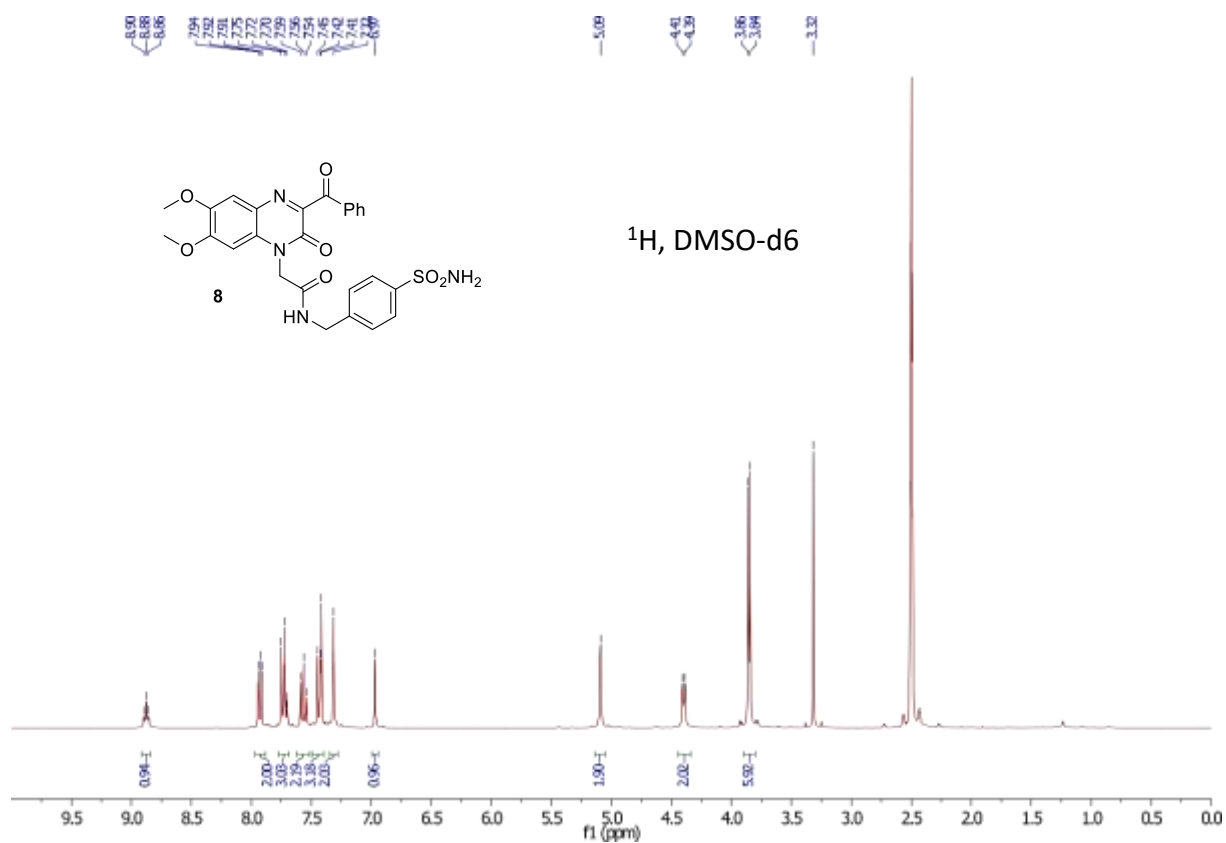


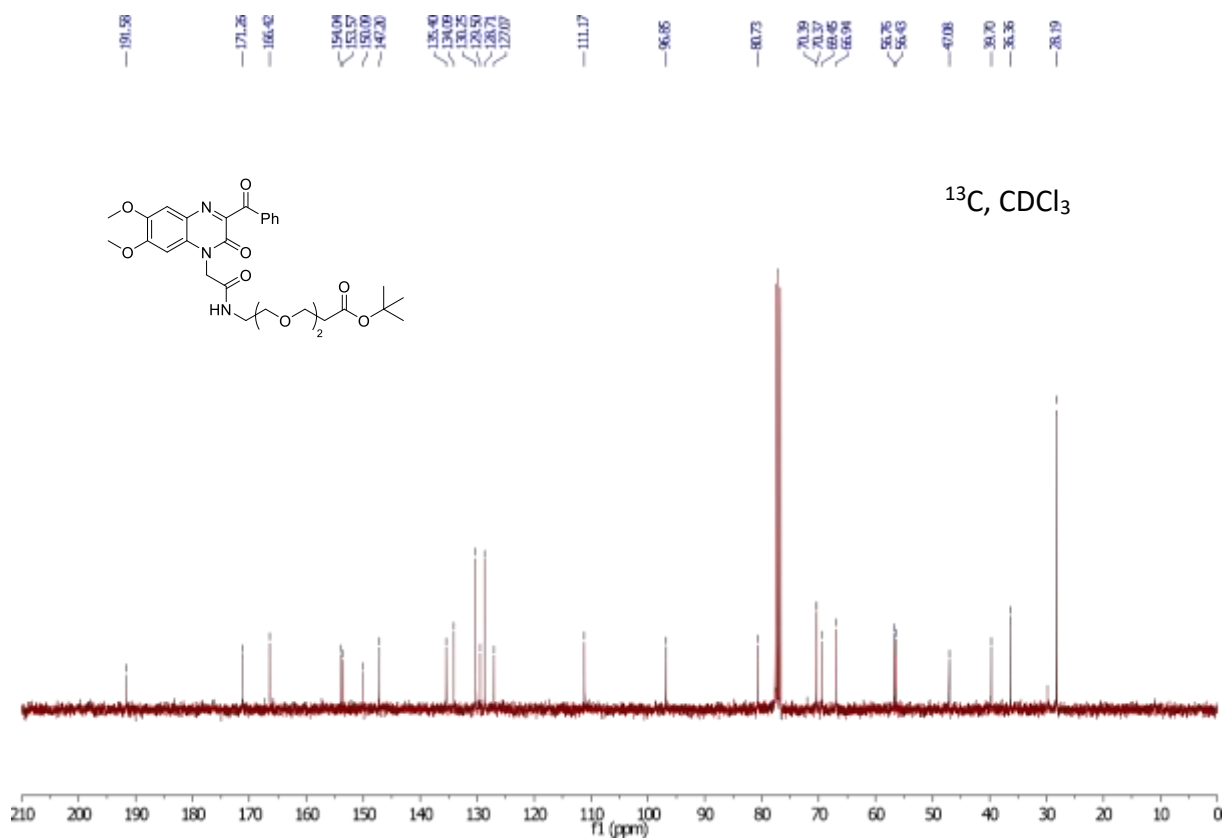
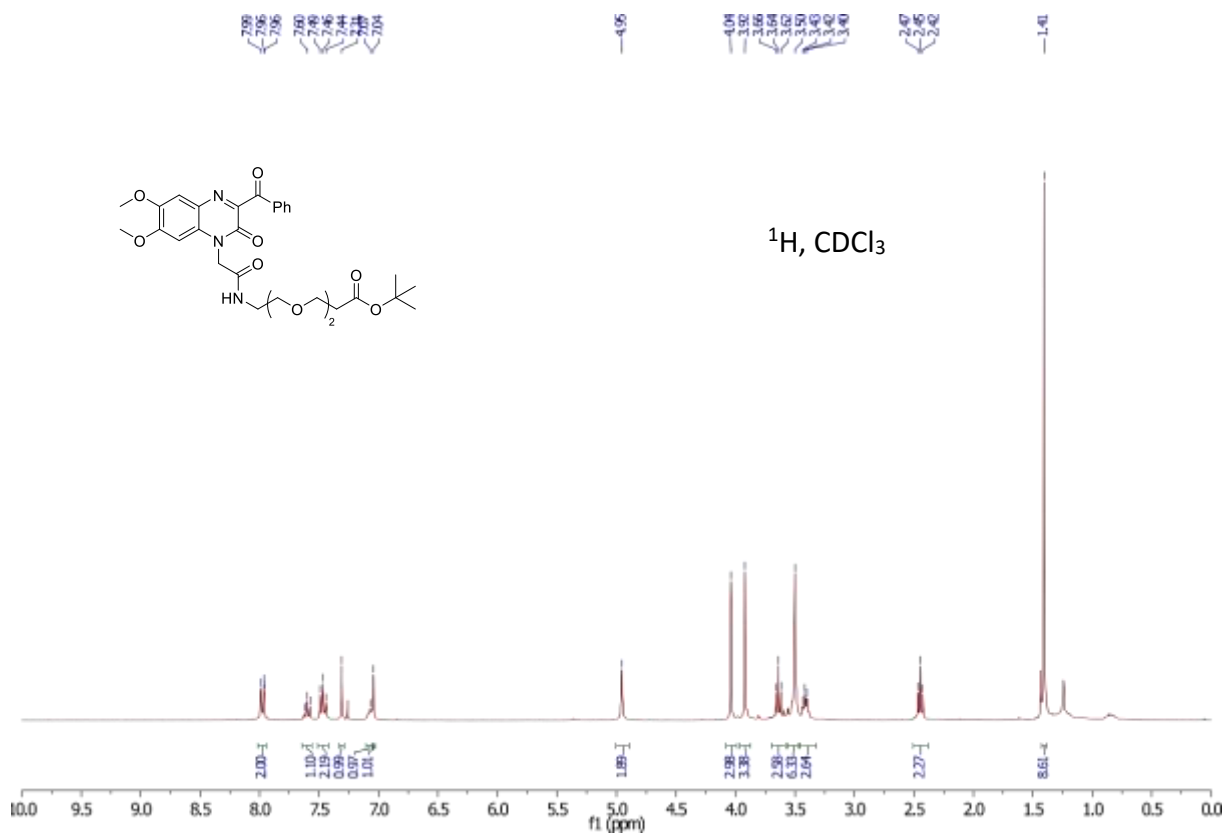


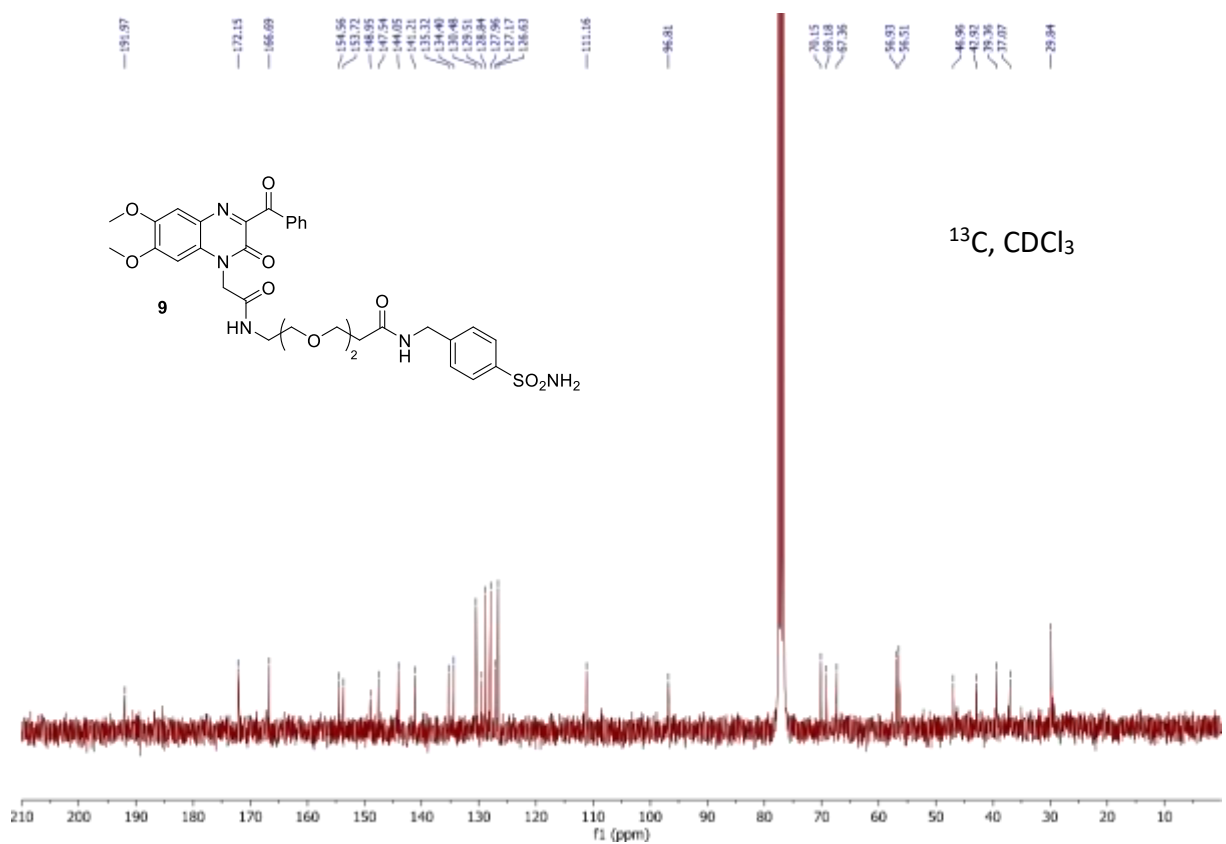
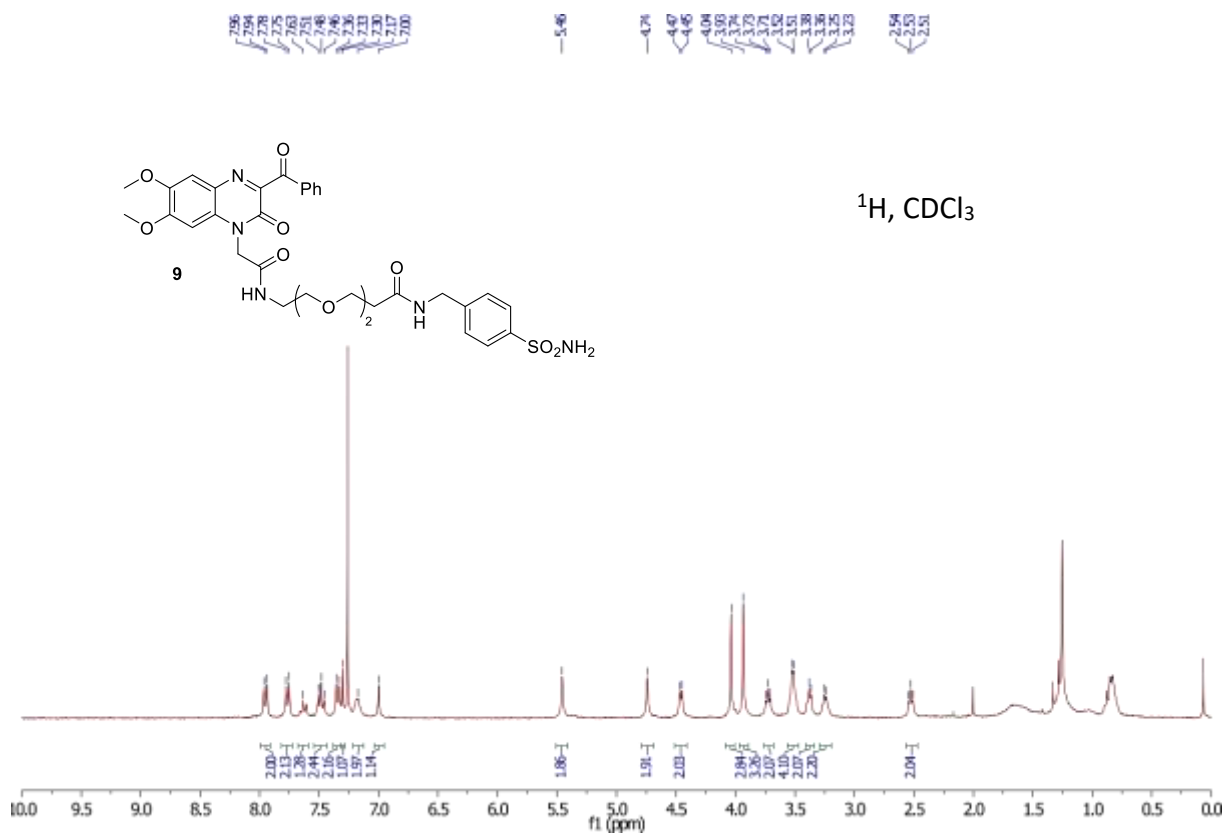


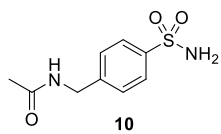




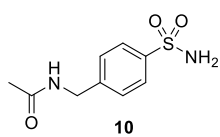
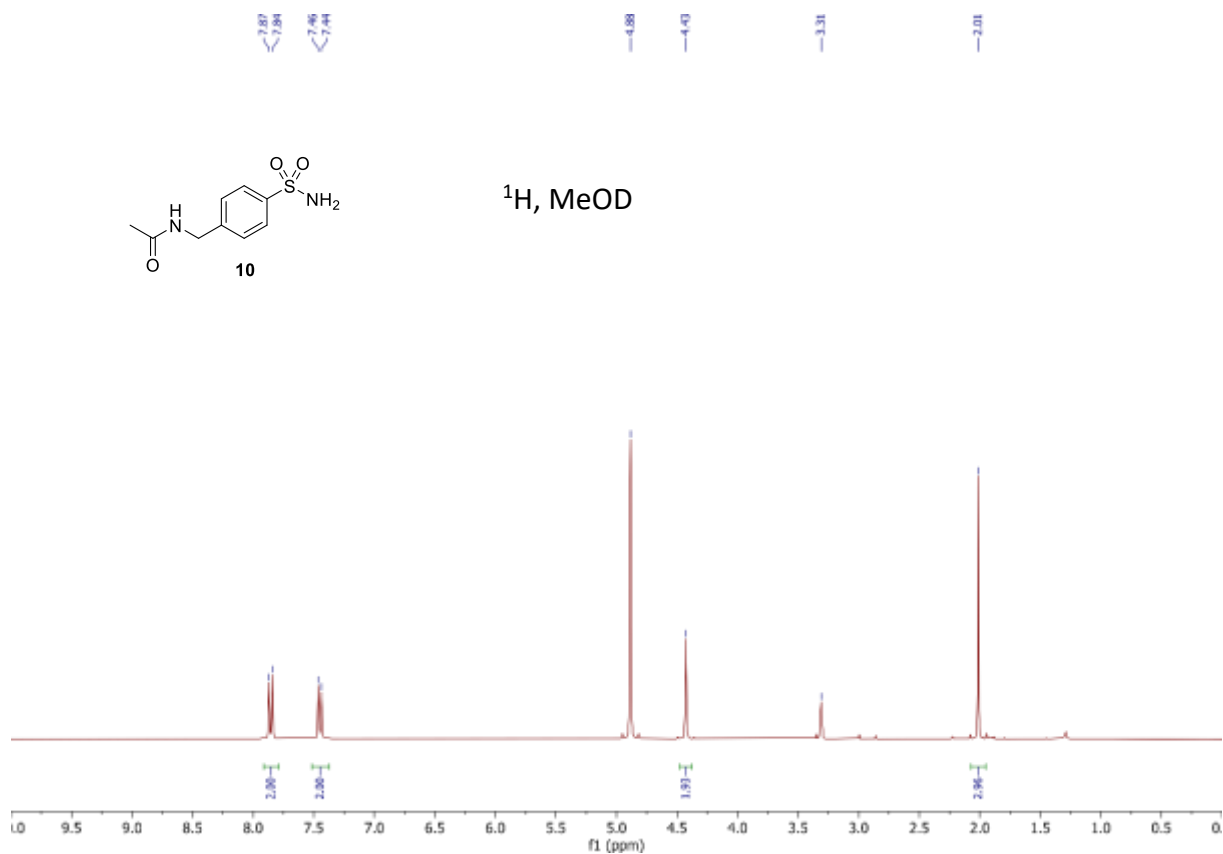




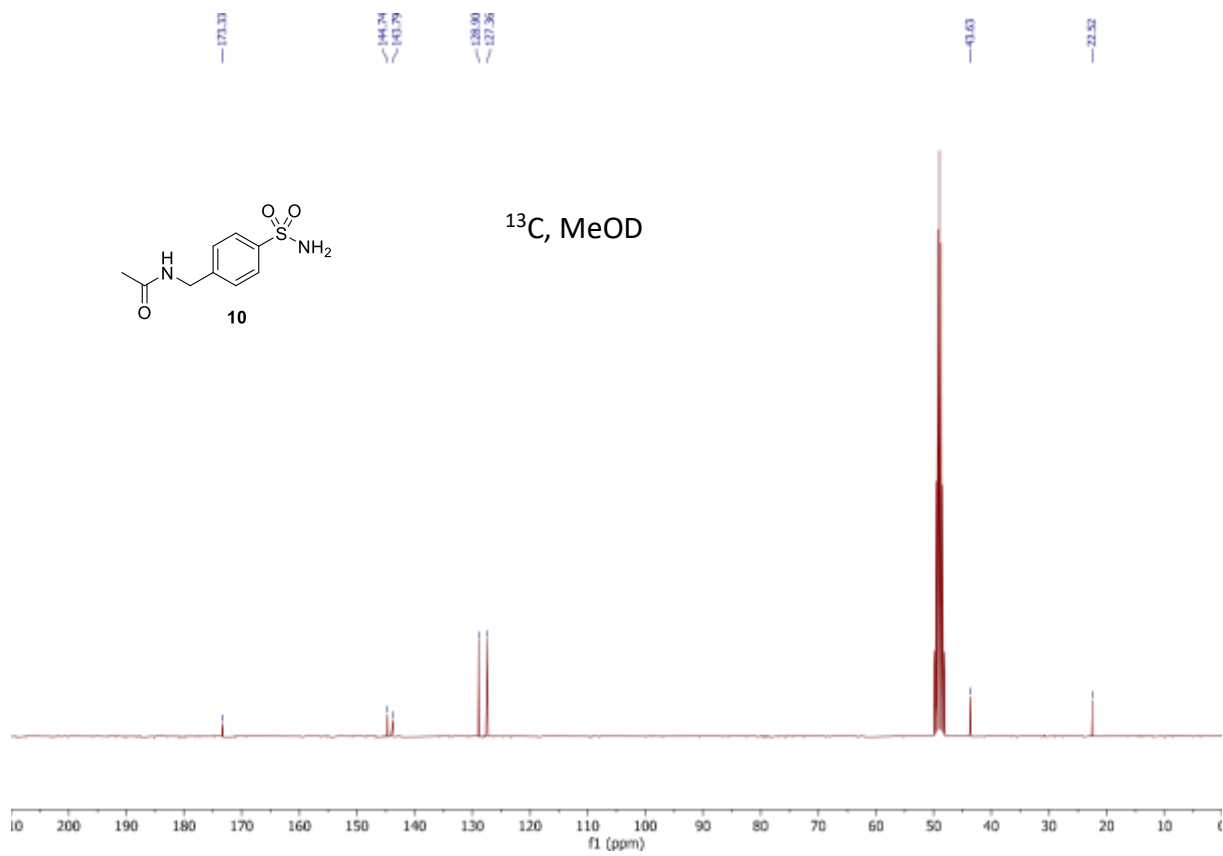


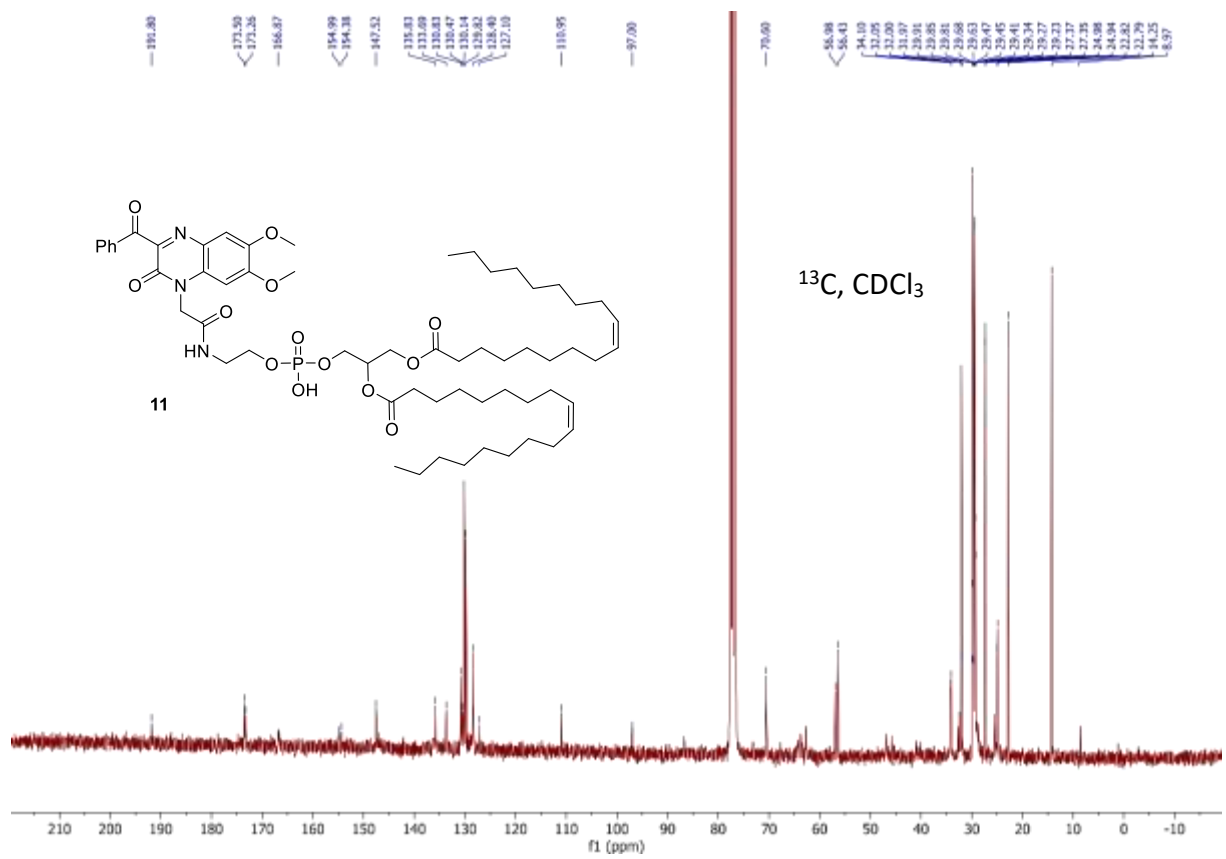
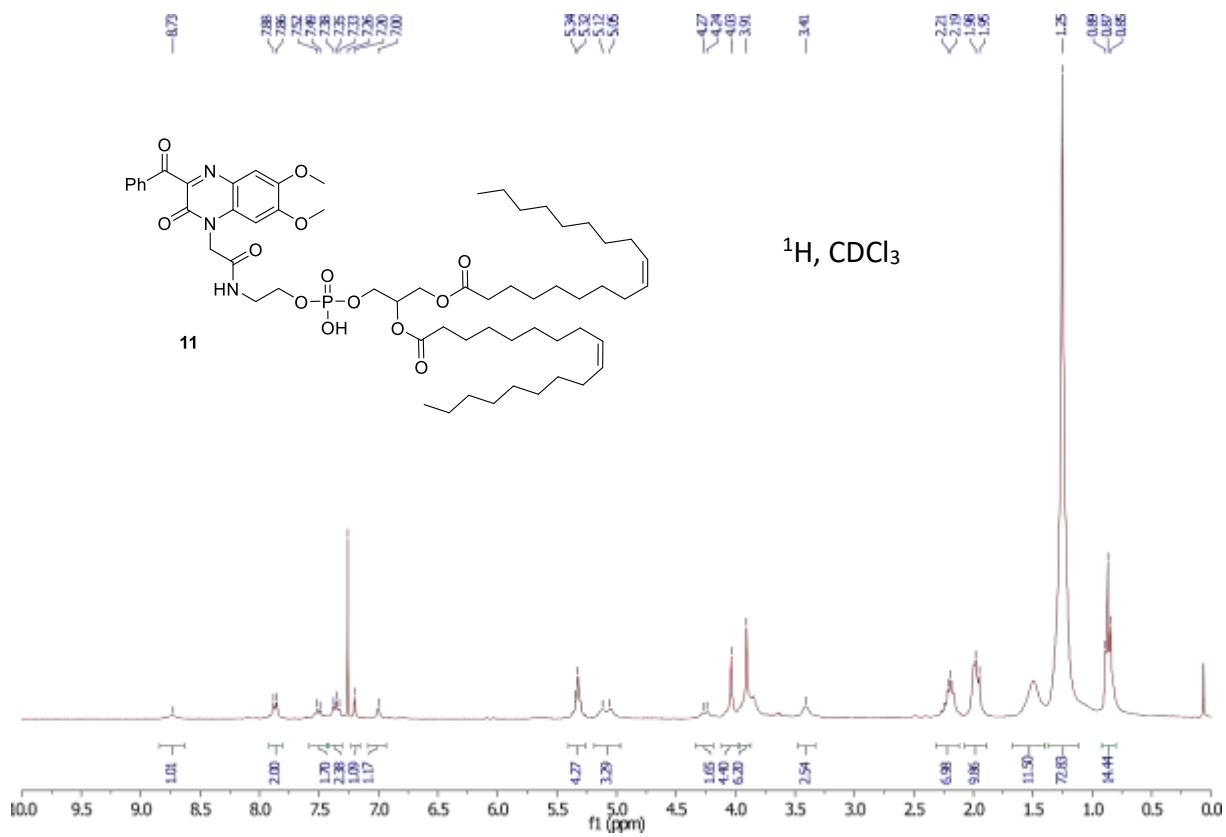


¹H, MeOD



¹³C, MeOD





III. Reaction of quinoxalinone **3** with NaBH₄

a) RP-HPLC study

To a 100 μ M solution of the quinoxalinone **3** in 3 mL of water was added NaBH₄ (0.1 mg, 10 equiv). RP-HPLC chromatograms were recorded before and after the addition of NaBH₄.

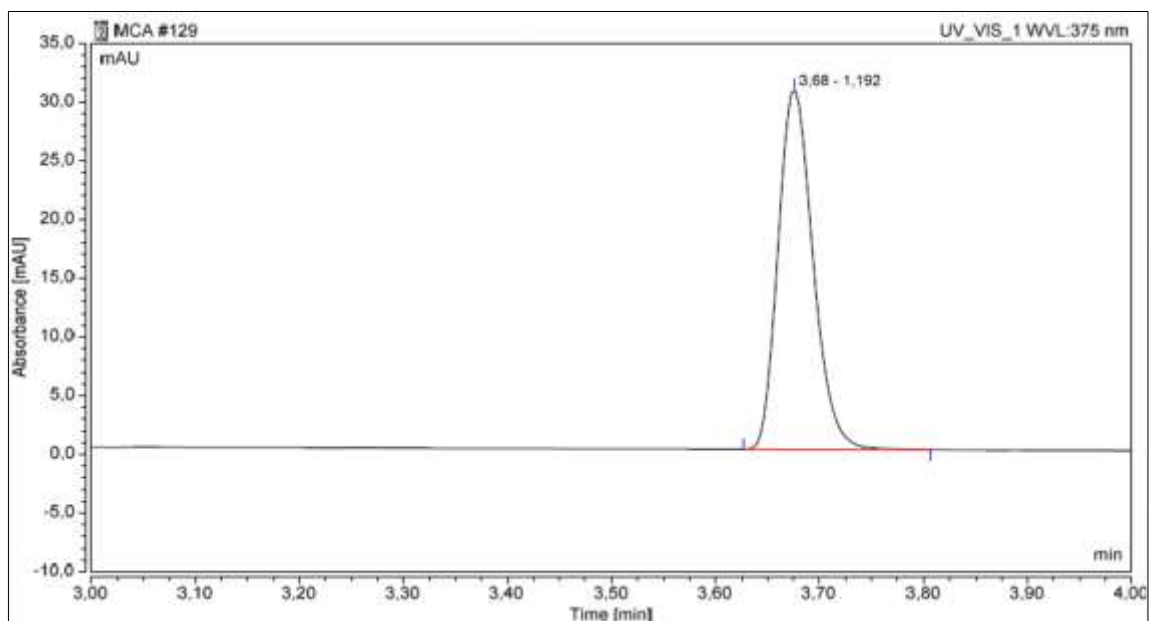


Figure S1 RP-HPLC chromatogram of compound **3** ($\lambda = 375$ nm).

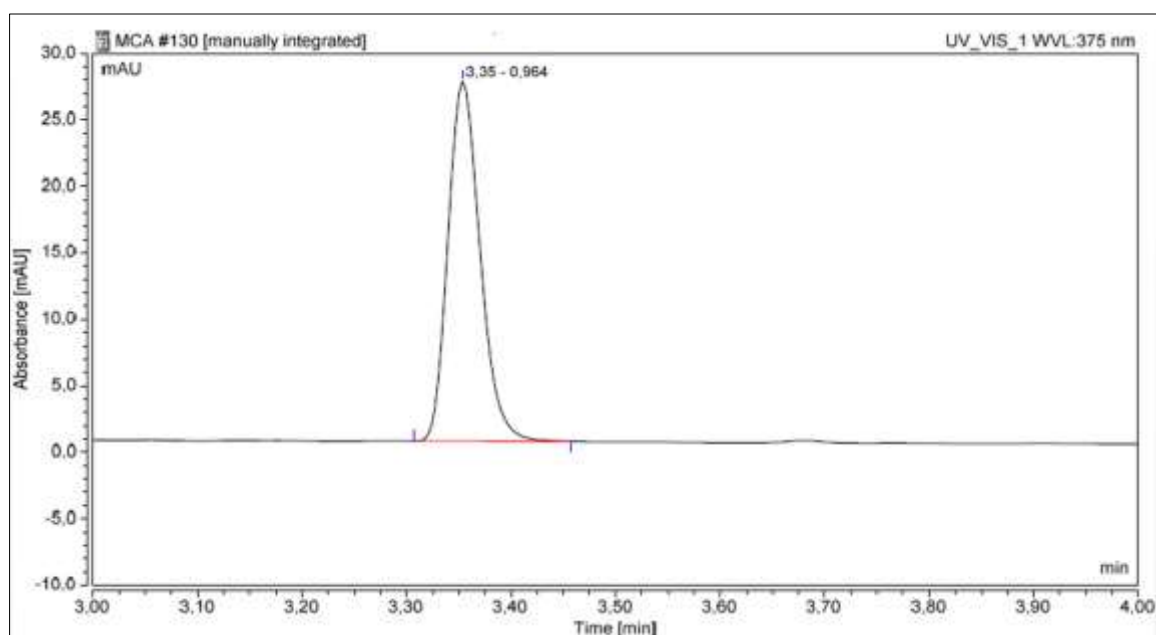


Figure S2 RP-HPLC chromatogram of compound **3** after addition of NaBH₄ ($\lambda = 375$ nm).

b) Fluorescence emission study

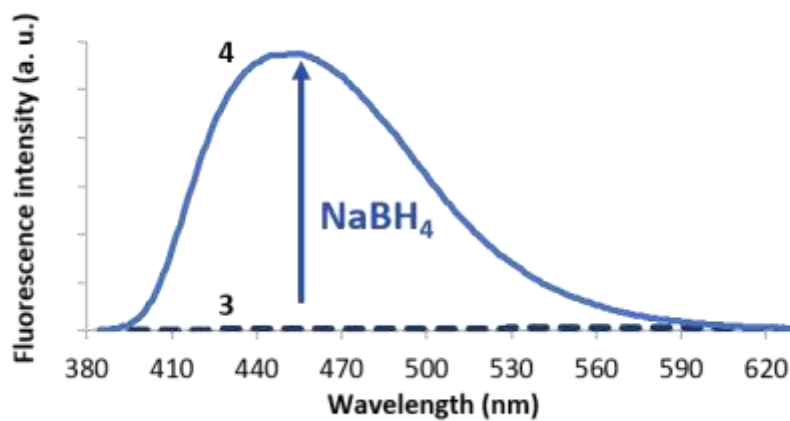


Figure S3 Fluorescence emission spectrum of quinoxalinone **3** (20 μ M, λ_{ex} 375 nm) in water at 25°C, before (dashed line) and after (blue line) the addition of NaBH₄ (10 equiv.) and shake for 5 minutes.

IV. Spectroscopic data for compounds **3** and **4**

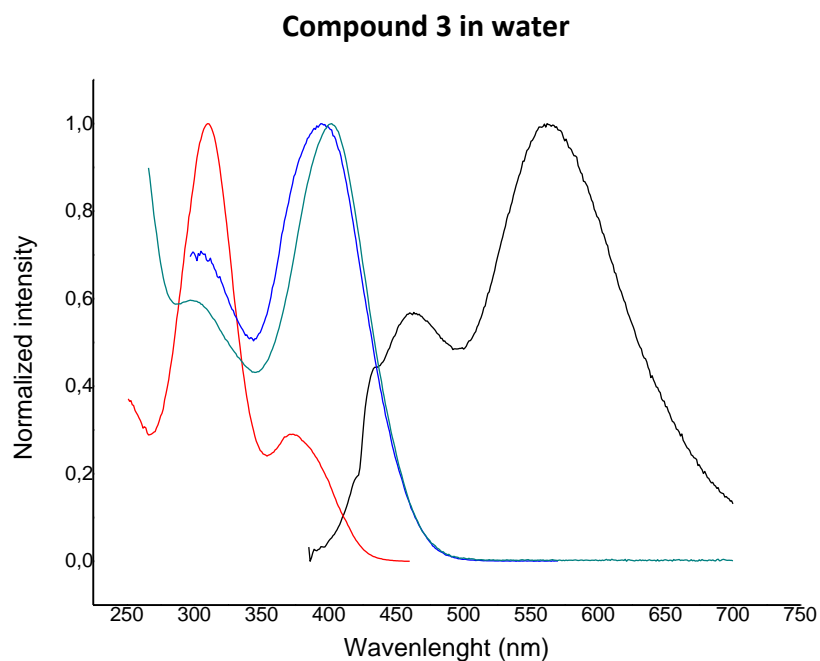


Figure S4 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375$ nm) and excitation (red trace, $\lambda_{\text{em}} = 460$ nm and green trace, $\lambda_{\text{em}} = 580$ nm) spectra for compound **3** at 25 °C in water.

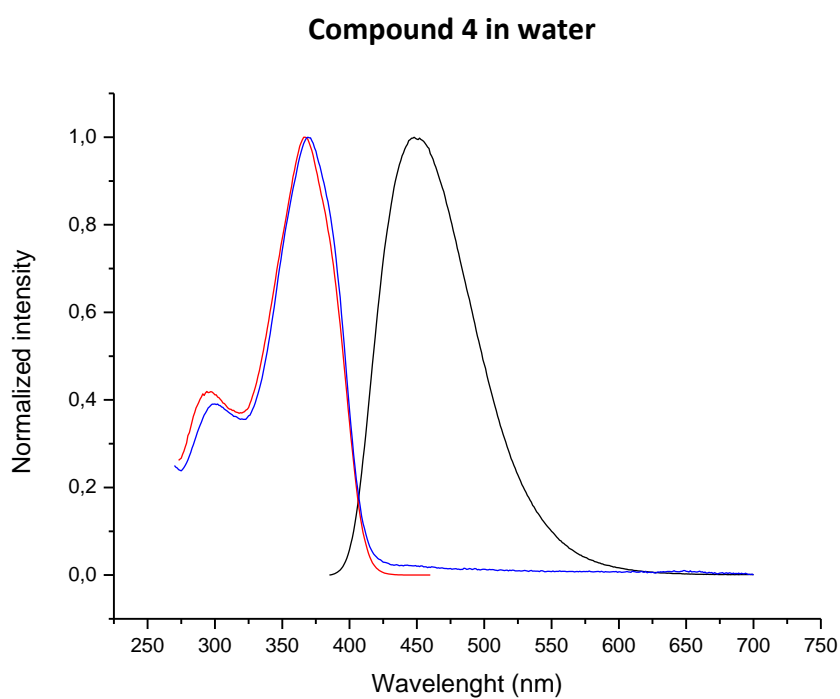


Figure S5 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375$ nm) and excitation (red trace, $\lambda_{\text{em}} = 470$ nm) spectra for compound **4** at 25 °C in water.

Compound 3 in PBS pH 7.4

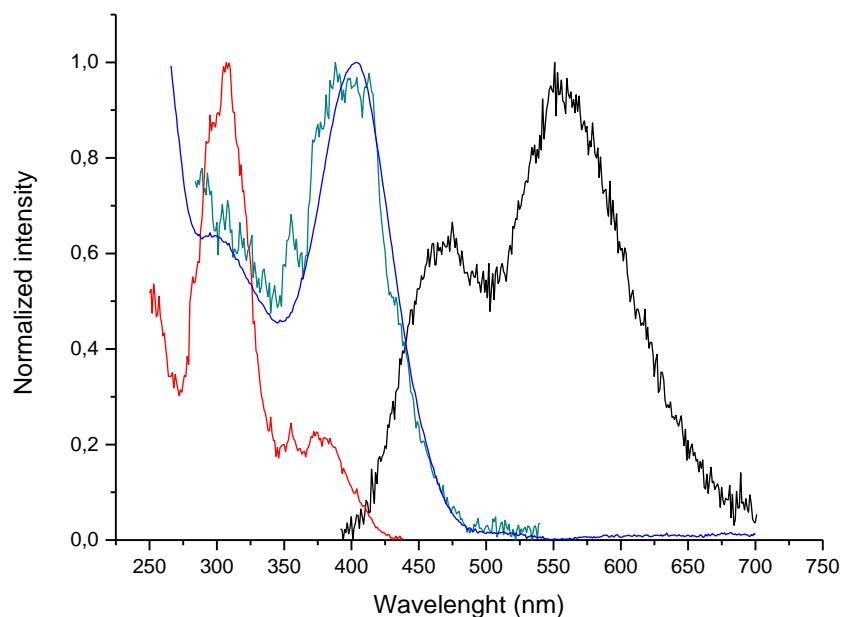


Figure S6 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375 \text{ nm}$) and excitation (red trace, $\lambda_{\text{em}} = 455 \text{ nm}$ and green trace, $\lambda_{\text{em}} = 555 \text{ nm}$) spectra for compound **3** at 25 °C in PBS pH 7.4.

Compound 4 in PBS pH 7.4

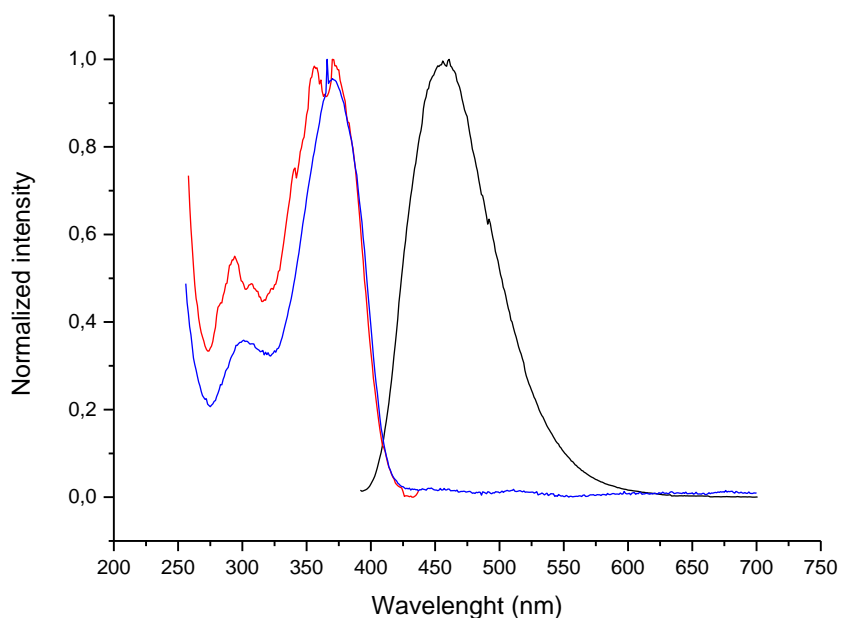


Figure S7 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375 \text{ nm}$) and excitation (red trace, $\lambda_{\text{em}} = 455 \text{ nm}$) spectra for compound **4** at 25 °C in PBS pH 7.4.

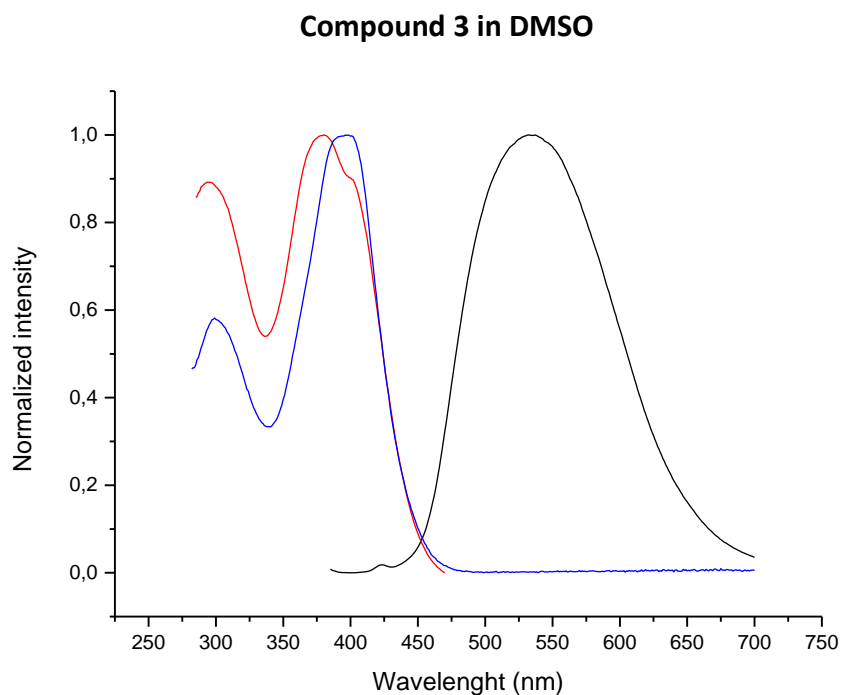


Figure S8 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375$ nm) and excitation (red trace, $\lambda_{\text{em}} = 480$ nm) spectra for compound **3** at 25 °C in DMSO.

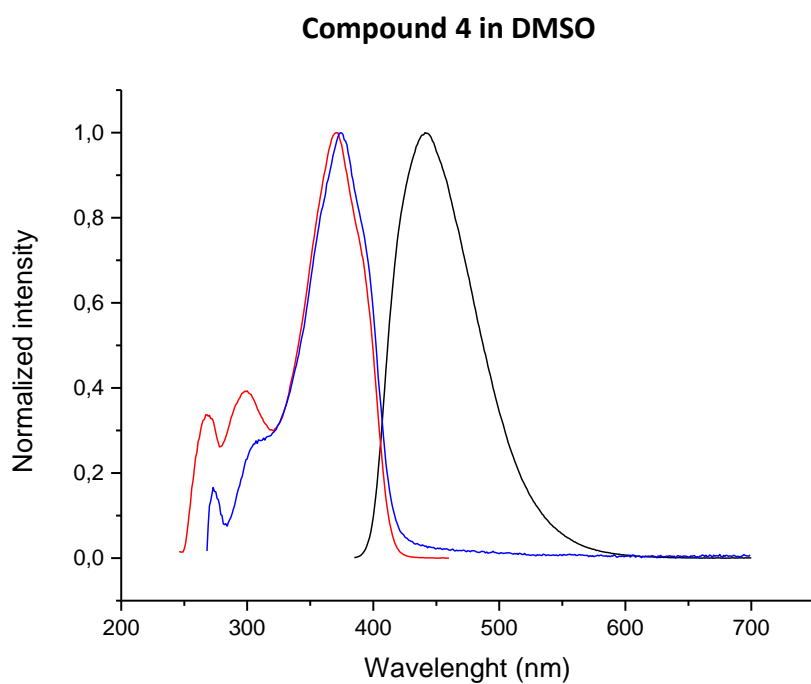


Figure S9 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375$ nm) and excitation (red trace, $\lambda_{\text{em}} = 470$ nm) spectra for compound **4** at 25 °C in DMSO.

Compound 7 in water

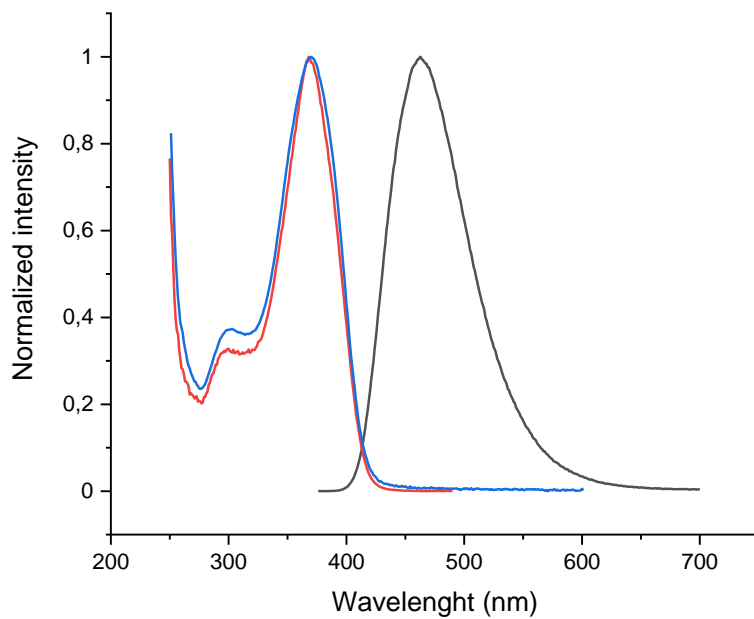


Figure S10 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375 \text{ nm}$) and excitation (red trace, $\lambda_{\text{em}} = 470 \text{ nm}$) spectra for compound **7** at 25 °C in water.

Compound 7 in PBS 7.4

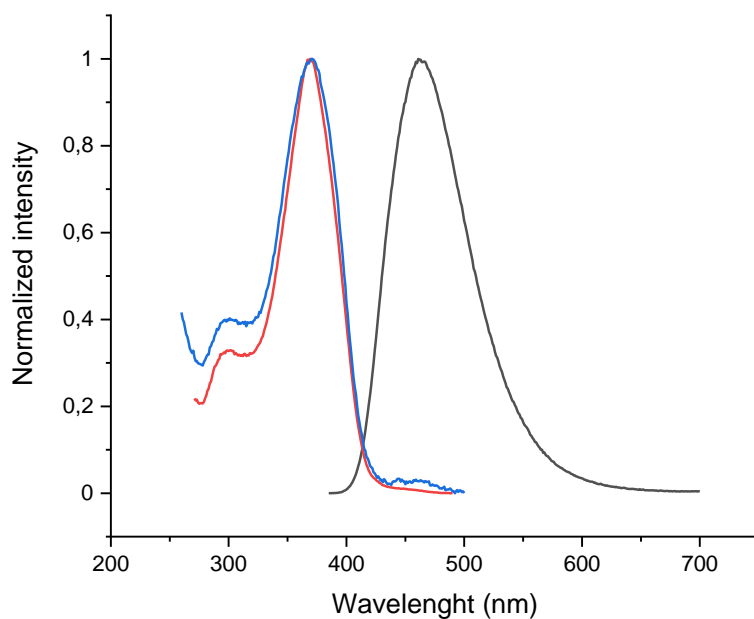


Figure S11 Normalized absorption (blue trace), emission (black trace, $\lambda_{\text{ex}} = 375 \text{ nm}$) and excitation (red trace, $\lambda_{\text{em}} = 470 \text{ nm}$) spectra for compound **7** at 25 °C in PBS pH 7.4.

Table S1 Spectral properties for compounds **3**, **4** and **7**

Cmpd	Solvent	$\lambda_{\text{abs max}}$ (nm)	$\lambda_{\text{em max}}$ (nm)	Stokes' shift (nm)	$E \text{ (M}^{-1} \text{ cm}^{-1}\text{)}$	Φ_{F}
3	PBS 7.4	403	557	154	11860	<0.01 ^a
	Water	402	562	160	11436	<0.01 ^a
4	PBS 7.4	370	456	86	11779	0.35 ^b
	Water	369	448	79	10504	0.37 ^b
7	PBS 7.4	370	461	91	n.d.	0.41 ^b
7	Water	370	463	93	n.d.	0.49 ^b

^aQuantum yield determined at 25 °C by the relative method using Lucifer Yellow ($\Phi_{\text{F}} = 0.21$ in water)² with excitation at 420 nm. ^bQuantum yield determined at 25 °C by the relative method using Quinine Sulfate ($\Phi_{\text{F}} = 0.55$ in sulfuric acid solution 0.5 M)¹ with excitation at 366 nm.

V. Photostability assay

Emission spectra were recorded before and after photostability assay performed with quinoxalinone **8** (100 μM in 3 mL of PBS pH 7.4) and its NaBH_4 -reduced form. The reduced form of quinoxalinone **8** was obtained by adding NaBH_4 (0.1 mg, 10 equiv) to a 100 μM solution of quinoxalinone **8** in PBS pH 7.4 (3 mL) contained in the spectroscopy cuvette. Quinoxalinone **9** and its reduced form were irradiated for 1h with the blue LED ribbon (λ_{max} 450 nm).

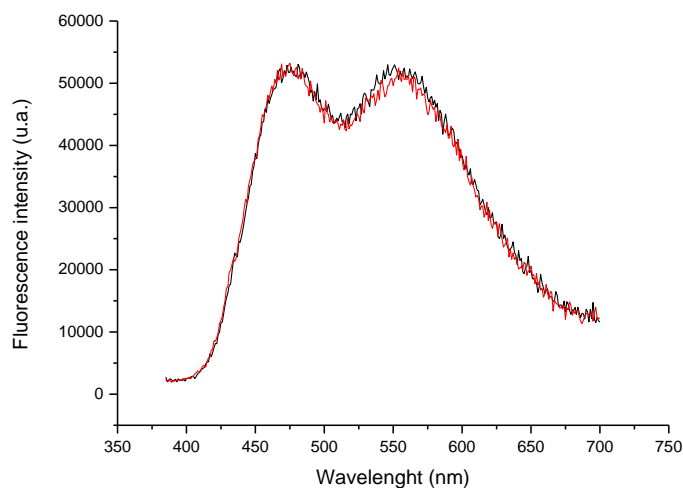


Figure S12 Emission spectra ($\lambda_{\text{ex}} = 375 \text{ nm}$) of quinoxalinone **8** before (black trace) and after (red trace) a 1h period of blue LED irradiation in PBS pH 7.4.

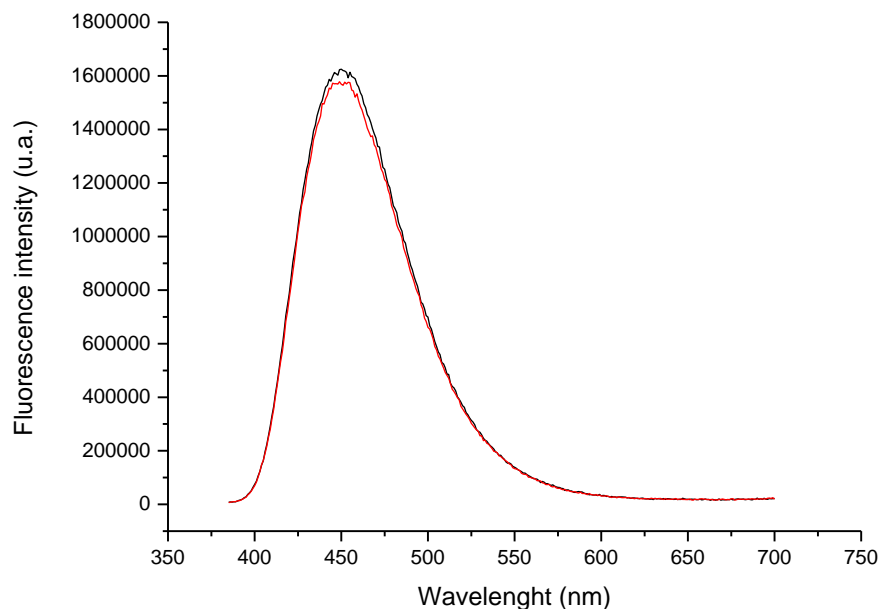


Figure S13 Emission spectra ($\lambda_{\text{ex}} = 375 \text{ nm}$) of the reduced form of quinoxalinone **8** by NaBH_4 before (black trace) and after (red trace) a 1h period of blue LED irradiation in PBS pH 7.4.

VI. labelling experiments of CA-II

a) Time-course labelling experiments of CA-II

a.1. With compounds **3** and **8** under visible blue LED strip (λ_{\max} 450 nm) irradiation

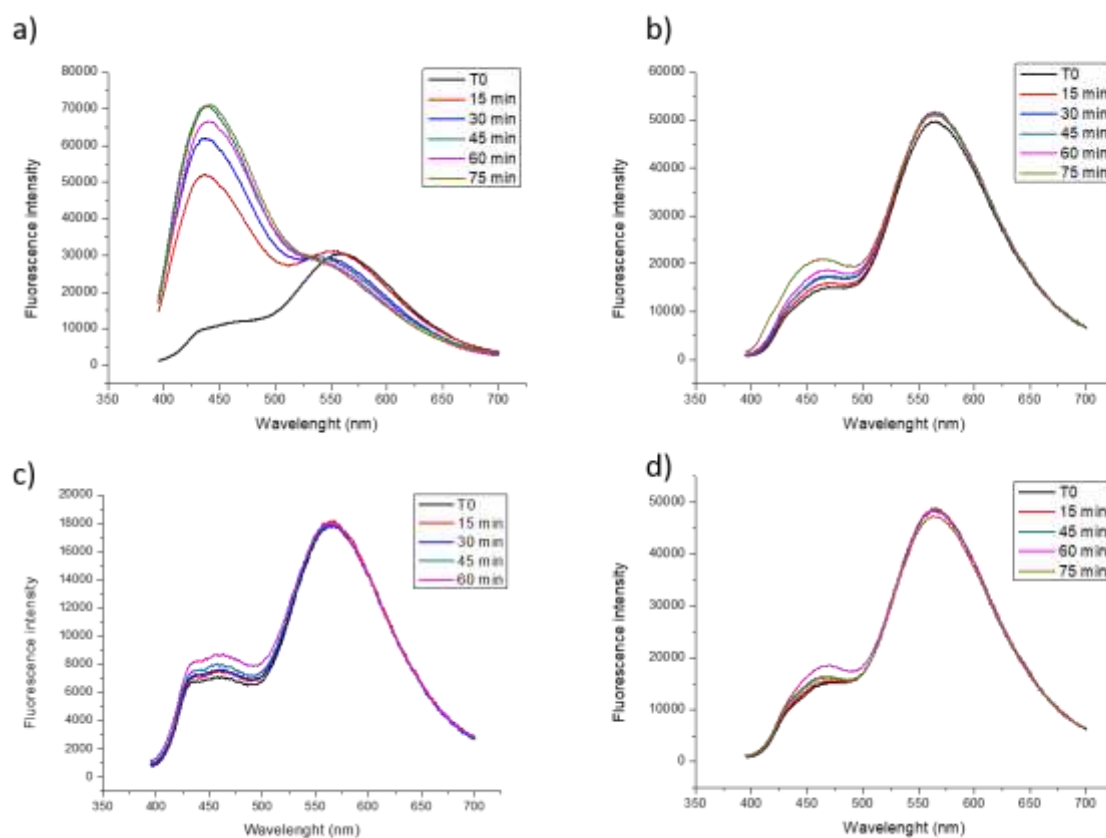


Figure S14 Time-course of fluorescence emission spectra of a mixture of CA-II (10 μM = 0.3 mg/mL) with different probes (10 μM , 1 eq.) (λ_{ex} 375 nm) in water at 25°C, under visible blue LED strip light (λ_{max} 450 nm) irradiation: (a) probe **8**; (b) 30 min pre-incubation with known CA-II inhibitor acetazolamide (100 μM , 10 eq.) before introducing probe **8**; (c) quinoxalinone **3**; (d) quinoxalinone **3** without CA-II.

a.2. With compounds **3** and **8** under near UV LED spotlight (405 nm) irradiation

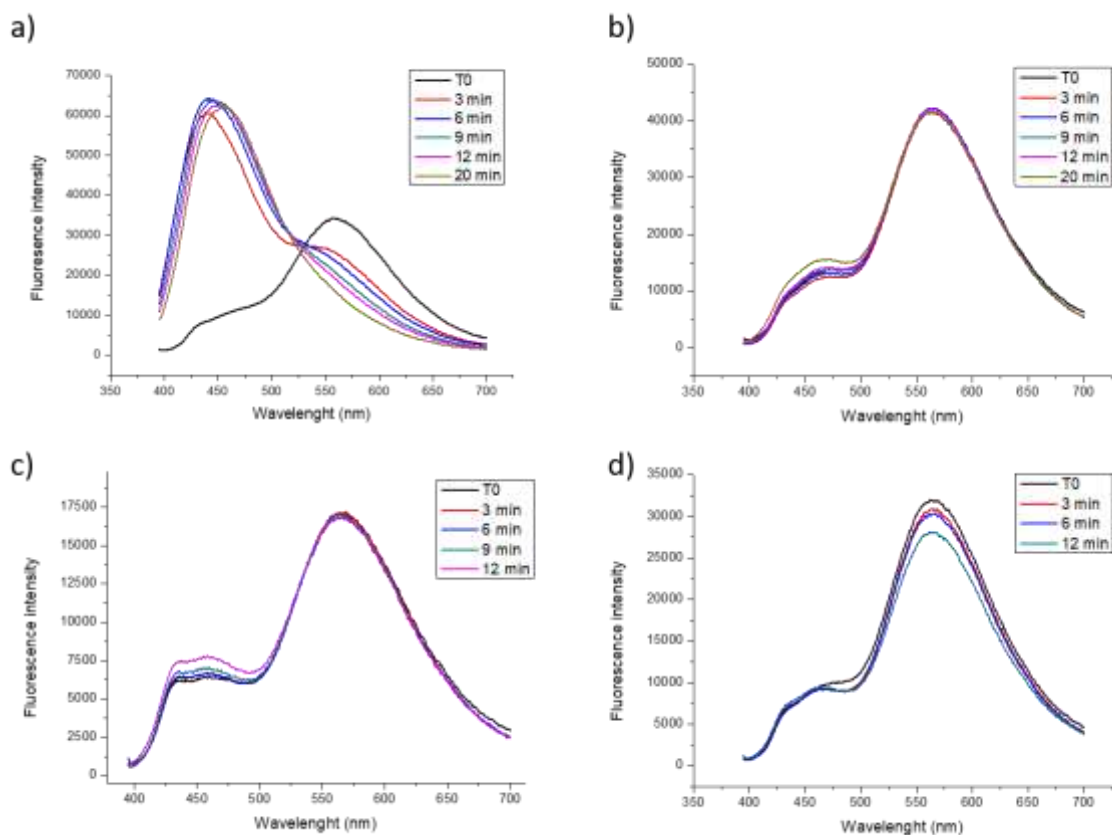


Figure S15 Time-course of fluorescence emission spectra of a mixture of CA-II (10 μ M = 0.3 mg/mL) with different probes (10 μ M, 1 eq.) (λ_{ex} 375 nm) in water at 25°C, under near UV LED spotlight (λ_{max} 405 nm) irradiation: (a) probe **8**; (b) 30 min pre-incubation with known CA-II inhibitor acetazolamide (100 μ M, 10 eq.) before introducing probe **8**; (c) quinoxalinone **3**; (d) quinoxalinone **3** without CA-II.

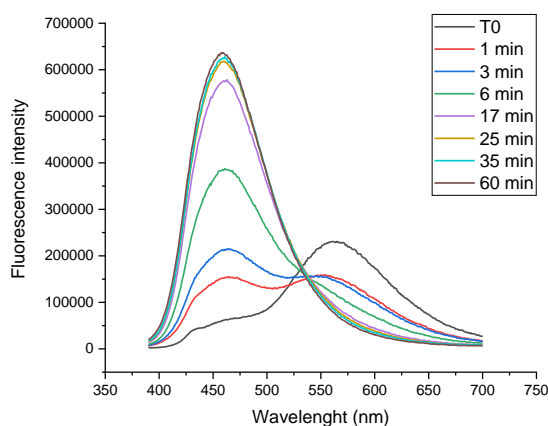


Figure S16 Time-course of fluorescence emission spectra of a mixture of CA-II (10 μM = 0.3 mg/mL) with probe **8** (10 μM , 1 eq.) (λ_{ex} 375 nm) in water at 25°C, under visible blue LED (λ_{max} 450 nm) irradiation.

b) RP-HPLC monitoring of the labelling of CA-II with compound **8** under visible blue LED (λ_{max} 450 nm) irradiation

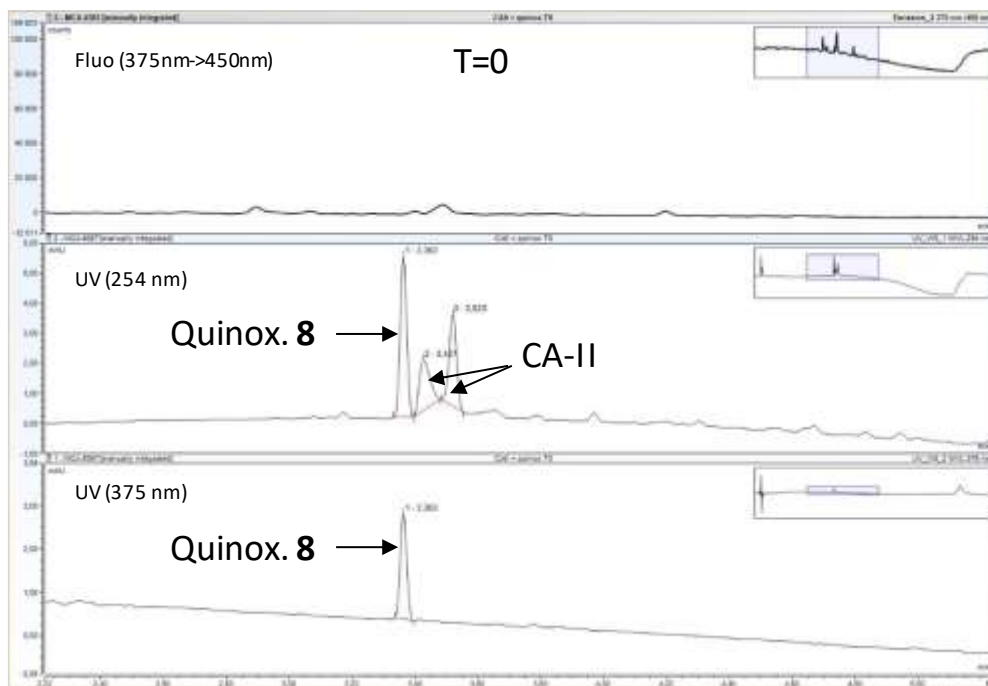


Figure S17 RP-HPLC chromatograms of a mixture of CA-II (10 μM) and quinoxalinone **8** (1 eq.) before irradiation ($t=0$), from top to bottom: fluorescence detection mode (λ_{ex} 375 nm, λ_{em} 450 nm); absorbance detection mode (254 nm); absorbance detection mode (375 nm).

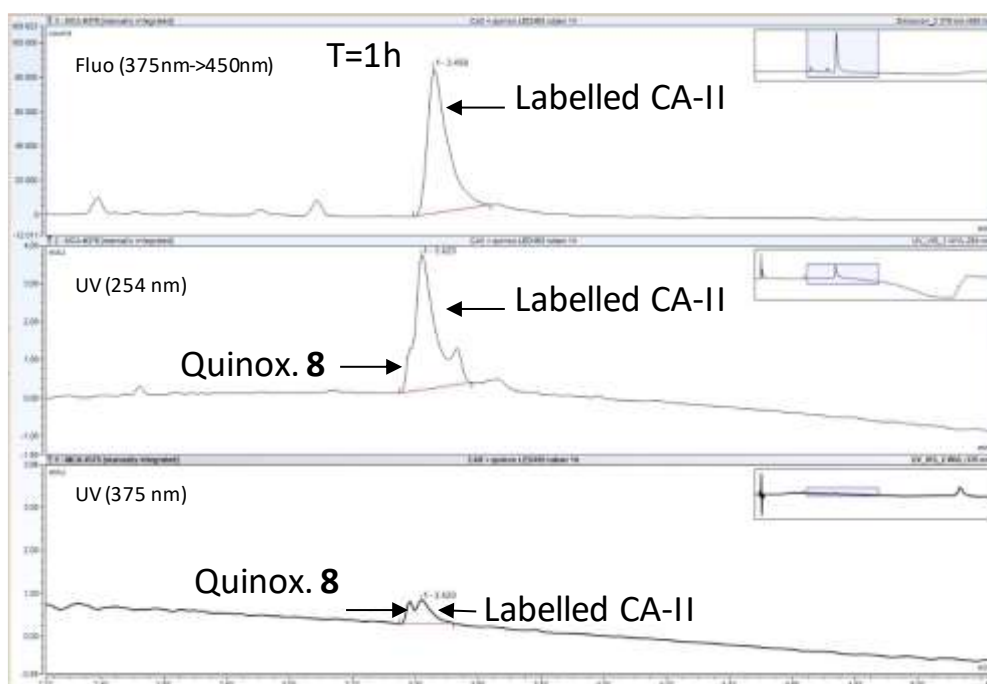


Figure S18 RP-HPLC chromatograms of the photoreaction between CA-II (10 μM) and quinoxalinone **8** (1 eq.) CA-II after 1h under visible blue LED irradiation (λ_{max} 450 nm), from top to bottom: fluorescence detection mode (λ_{ex} 375 nm, λ_{em} 450 nm); absorbance detection mode (254 nm); absorbance detection mode (375 nm).

c) Labelling efficiency of CA-II by quinoxalinone **8**

To estimate the labelling efficiency of CA-II by quinoxalinone **8**, a calibration curve was performed to convert the fluorescence intensity signal into quinoxalinone product concentration. To this end, the quinoxalinone photoproduct **7** was used as the model crosslinked photoproduct. The calibration curve was performed by determining the fluorescence emission intensity (λ_{ex} 375 nm, λ_{em} 450 nm) at different concentrations of compound **7** (0.1-10 μM) in PBS pH 7.4. Experimentally, a stock solution of compound **7** in DMSO (1 mM) was used to prepare solutions in PBS pH 7.4 (<1% DMSO) at different concentrations (0.1-10 μM)

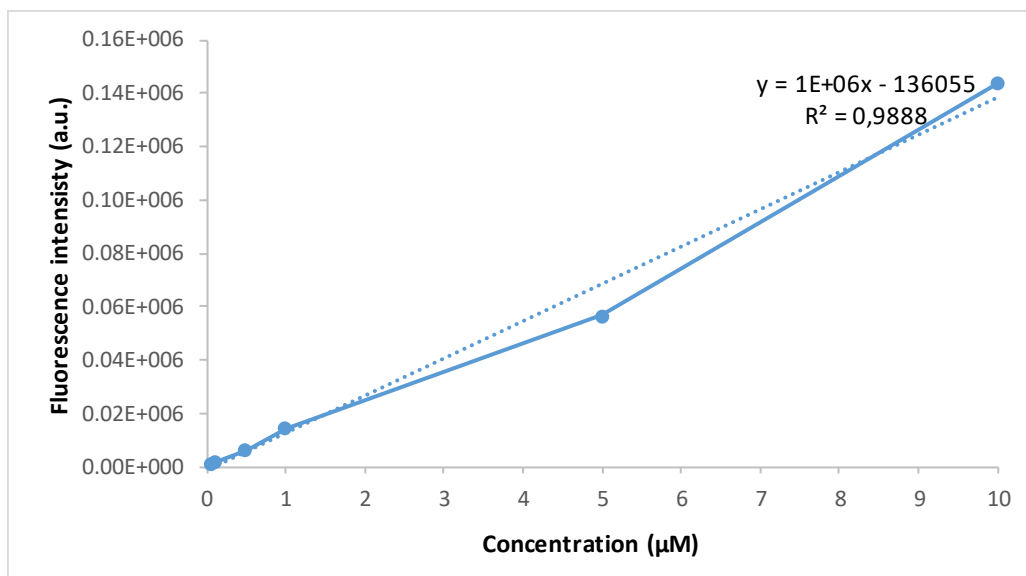


Figure S19 Fluorescence intensity for quinoxalinone **7** plotted against concentration (λ_{ex} 375 nm, λ_{em} 450 nm).

The fluorescence emission observed at 450 nm of a solution of CA-II (10 μM) in the presence of quinoxalinone **8** after 2 h under visible blue LED irradiation (λ_{max} 450 nm) was found to be 85175 a.u., which corresponds to a labelling yield of 2.2%.

VII. CA-II inhibitory activity assay

Inhibitory activity was determined spectrophotometrically using Bovine CA-II and *p*-nitrophenyl acetate (*p*NPA) as a substrate. In fact, *p*NPA is hydrolyzed by CA-II to form *p*-nitrophenol (*p*NP) which was observed spectrophotometrically at 348 nm.

Reaction mixture contained 60 μ L of Tris buffer (pH 7.6, 50 mM), 10 μ L (0.5 mM) of derivative **3**, **8** or **11** in 1% DMSO and 10 μ L CA-II (7 μ M, 0.21 g/L in Tris buffer) per well. Contents were mixed and pre-incubated at 25 °C for 10 min. A 20 μ L stock solution of *p*NPA (freshly prepared stock solution at 3 mM with <5% DMSO in Tris buffer) was added per well to achieve 0.6 mM concentration per well. Total reaction volume was made to 100 μ L. Plates were read at 348 nm each minute for 30 minutes. CA-II activity is reported **Figure S11**.

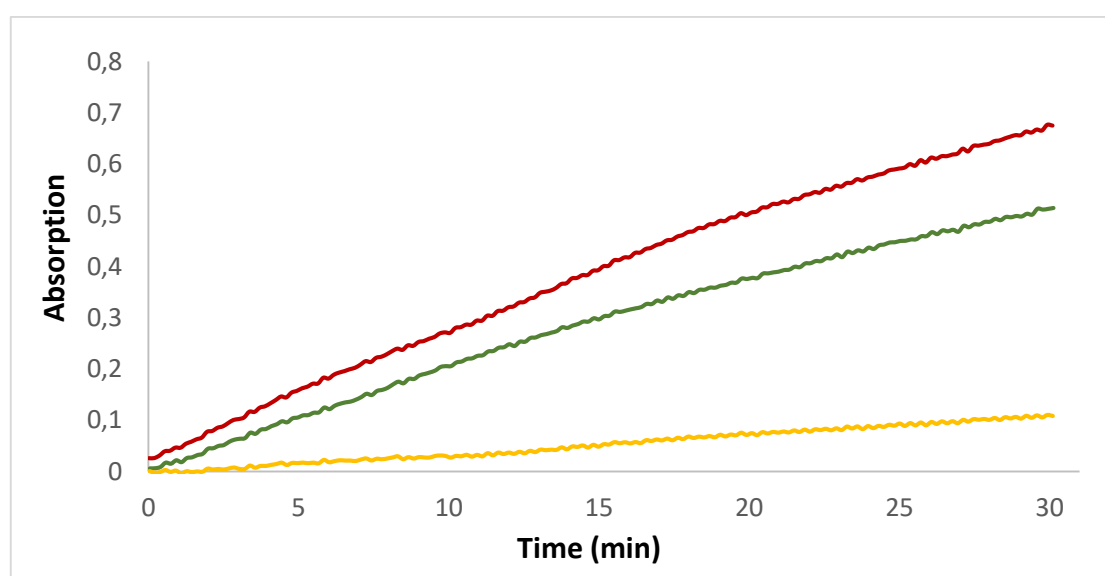


Figure S20 CA-II activity without inhibitor (red trace) or in pre-incubation with quinoxalinone **3** (green trace), quinoxalinone **8** (yellow trace).

For IC_{50} measurement, the same protocol has been used, except that 7 different concentrations were used for each compound. The results are shown in Table S2, based on the IC_{50} concentration-response curves which were made by tracing the slope of CA-II activity depending on \log_{10} (concentration) (**Figure S12**).

Compound	10	8	9
IC_{50} (μ M \pm SEM; $n = 3$)	1.9 ± 0.22	1.19 ± 0.27	1.46 ± 0.13

Table S2 IC_{50} values of different inhibitors toward CA-II. Results reported are mean of three independent experiments (\pm SEM)

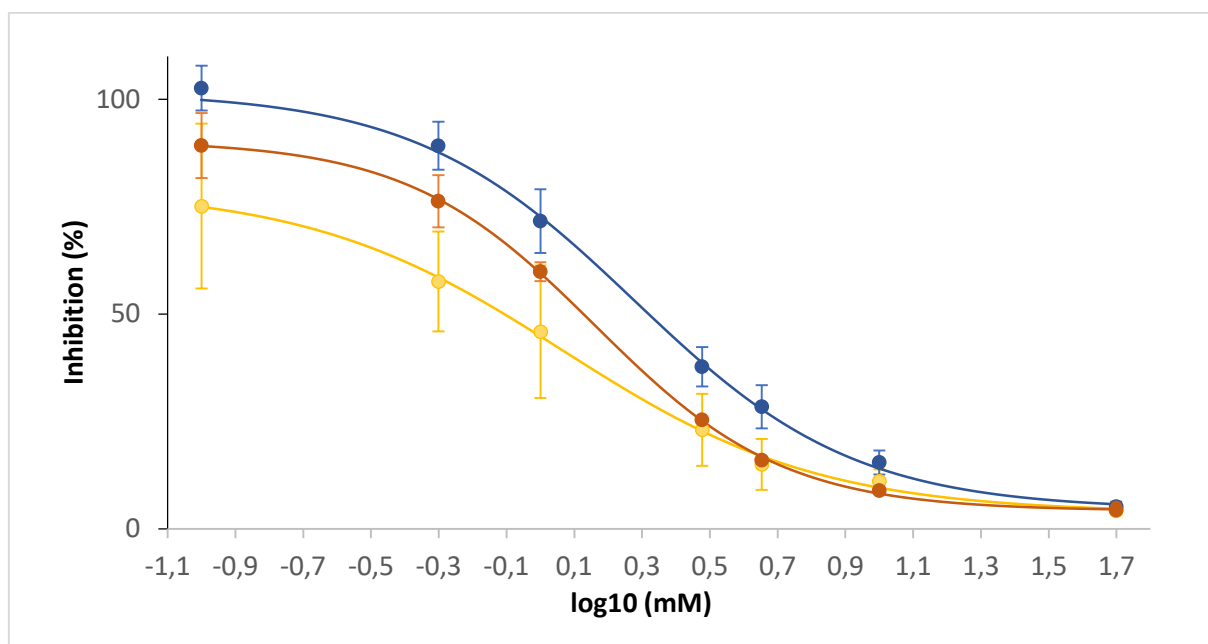
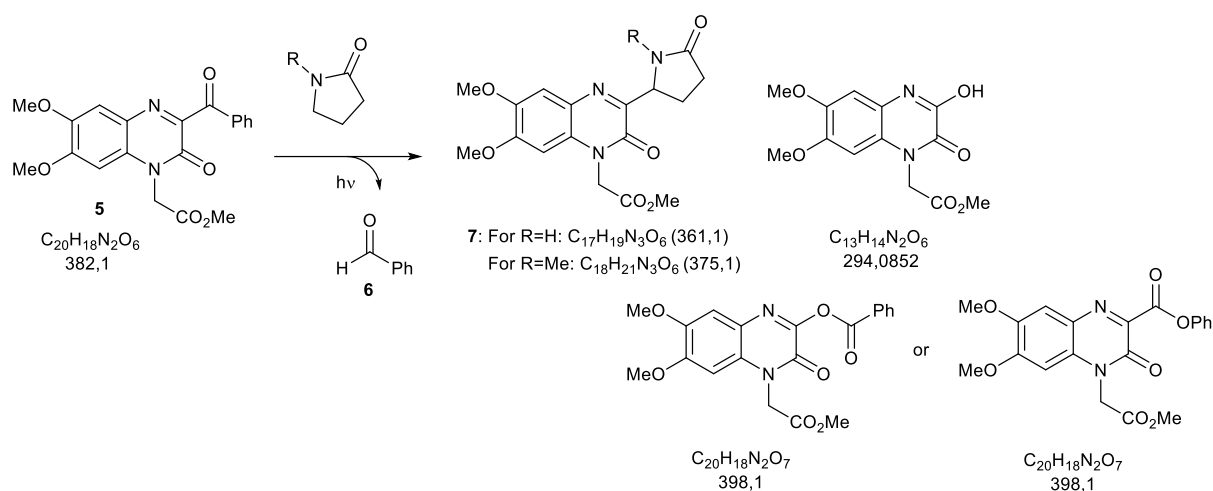


Figure S21 IC₅₀ concentration-response curves (mean ± SD; n = 3) obtained for different inhibitors: sulfonamide **10** (blue trace), quinoxalinone **8** (yellow trace) and quinoxalinone **9** (orange trace).

VIII. Photoreactions between pyrrolidinone (or N-methylpyrrolidinone) and quinoxalinone **5** or benzophenone

a) Photoreaction between quinoxalinone **5** and pyrrolidinone or N-methylpyrrolidinone (NMP)



Procedures:

- Without water: Quinoxalinone **5** (1 mg, 3 μ mol) in 2-pyrrolidinone (200 mg, 2.35 mmol, 900 equiv) was irradiated in a glass tube with a visible blue LED strip light lamp (λ_{max} 450 nm) for 2 h. The reaction was monitored by analytical RP-HPLC-MS.
- With water : Quinoxalinone **5** (1 mg, 3 μ mol) in 2-pyrrolidinone (200 mg, 2.35 mmol, 900 equiv) and water (100 μ L) was irradiated in a glass tube with a visible blue LED strip light lamp (λ_{max} 450 nm) for 2 h. Similar HPLC profiles were obtained (data not shown).

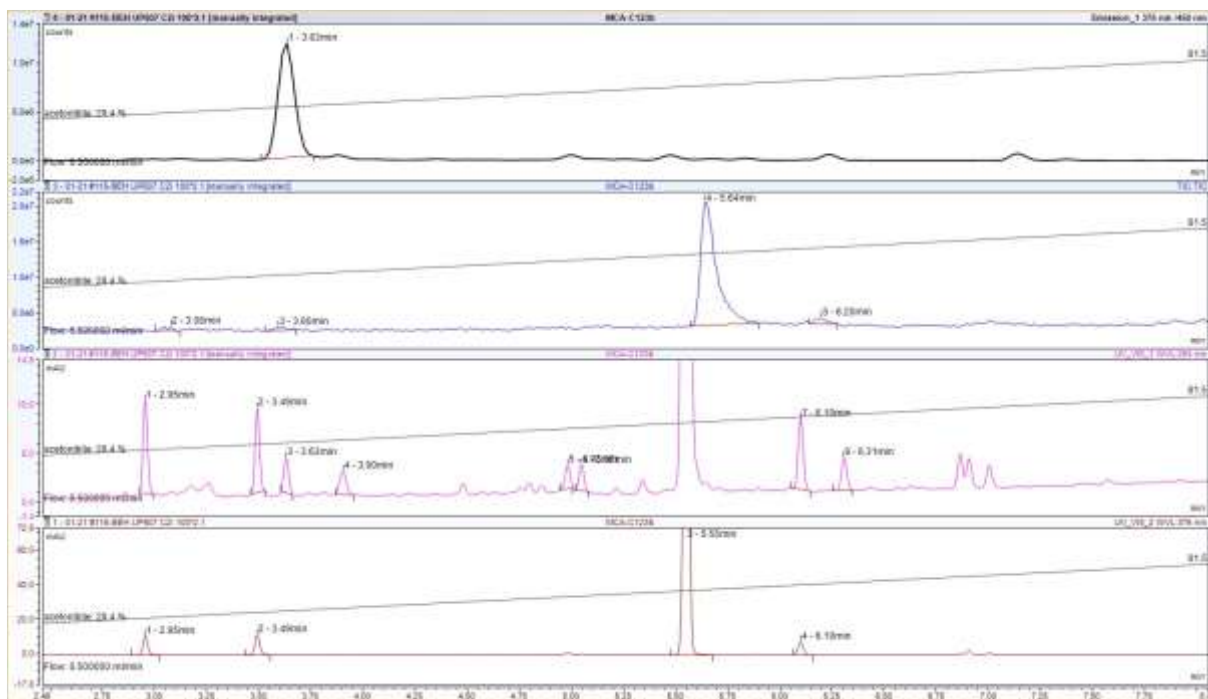


Figure S22 RP-HPLC-MS chromatograms of the photoreaction between quinoxalinone **5** and pyrrolidinone after 2h under visible blue LED irradiation (λ_{\max} 450 nm), from top to bottom: fluorescence detection mode (λ_{ex} 375 nm, λ_{em} 450 nm); TIC detection mode; absorbance detection mode (254 nm); absorbance detection mode (375 nm).

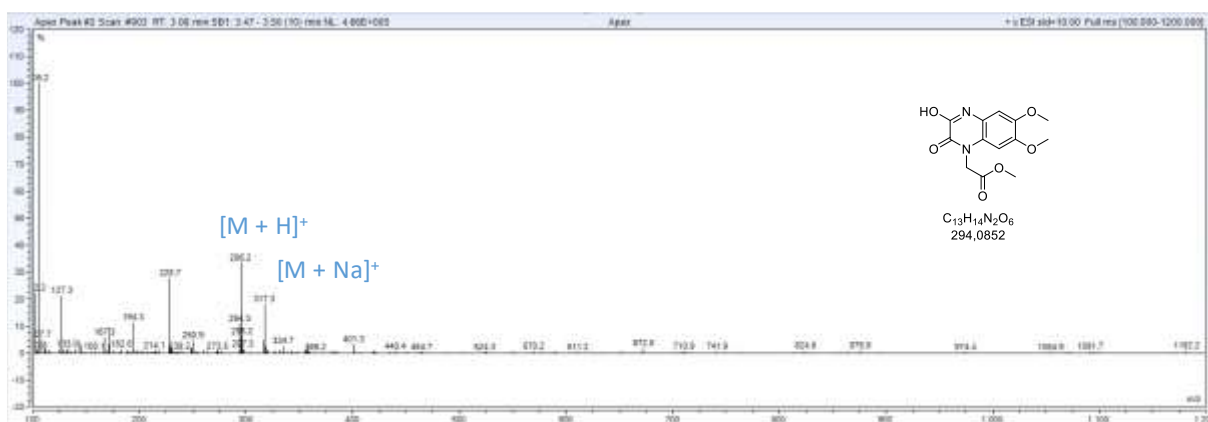


Figure S23 ESI mass spectrum associated to the peak at $t_{R(\text{MS})} = 3.08$ min (plausible chemical structure).

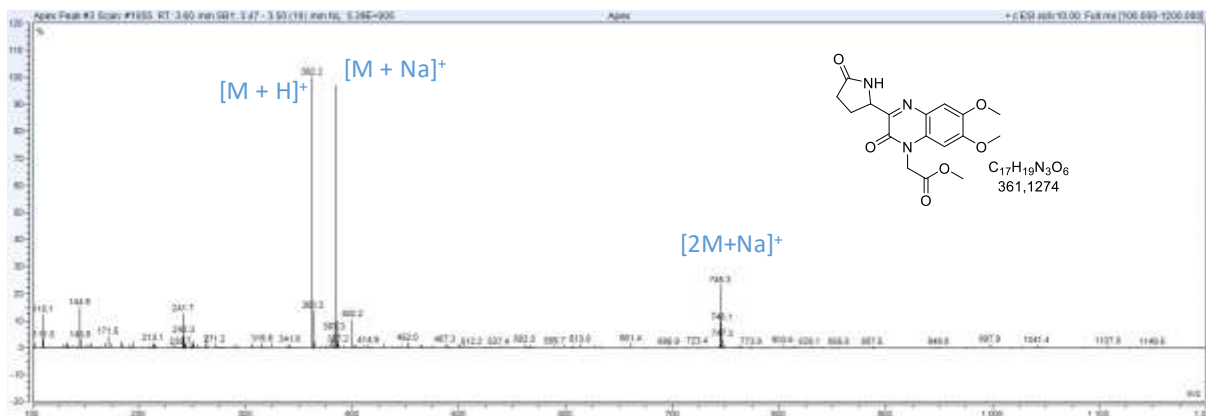


Figure S24 ESI mass spectrum associated to the peak at $t_{R(MS)} = 3.60$ min (structure confirmed with an authentic standard).

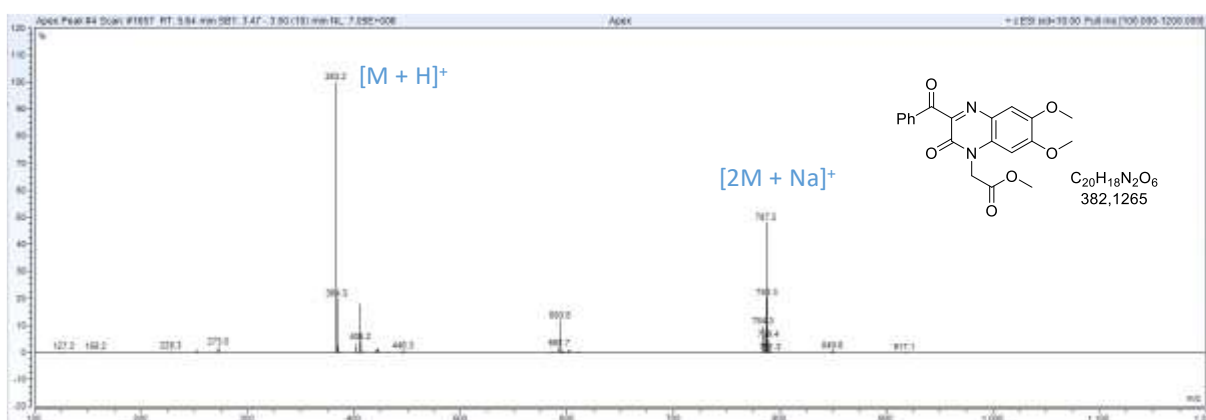


Figure S25 ESI mass spectrum associated to the peak at $t_{R(MS)} = 5.64$ min (structure confirmed with an authentic standard).

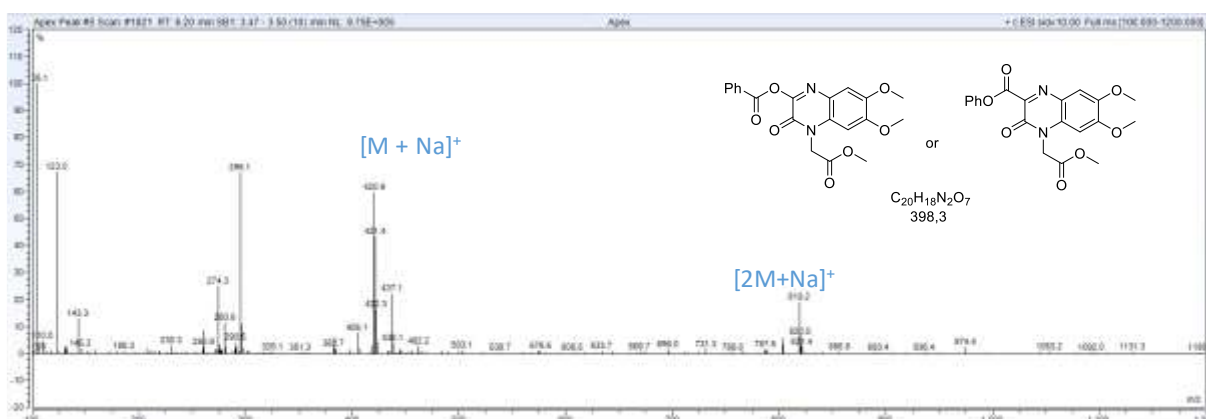


Figure S26 ESI mass spectrum associated to the peak at $t_{R(MS)} = 6.20$ min (plausible chemical structures).

b) Photoreaction between quinoxalinone **5** and N-methylpyrrolidinone NMP

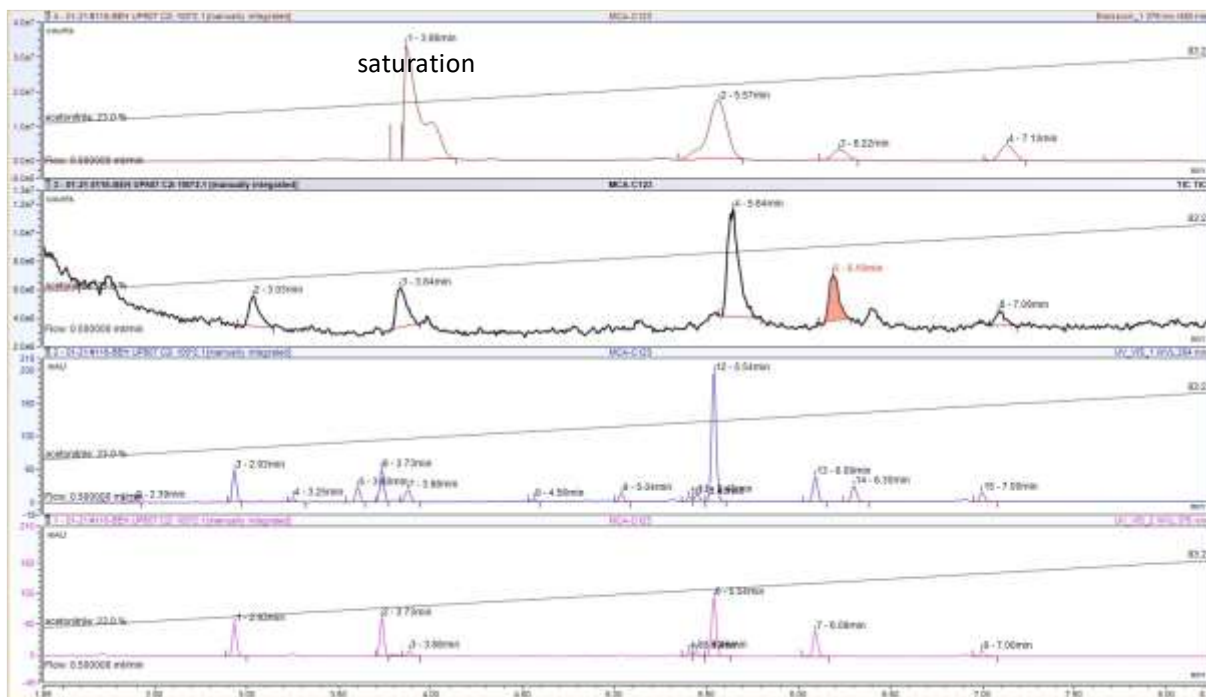


Figure S27 RP-HPLC-MS chromatograms of the photoreaction between quinoxalinone **5** and N-methylpyrrolidinone (NMP) after 2 h under visible blue LED irradiation (λ_{\max} 450 nm), from top to bottom: fluorescence detection mode (λ_{ex} 375 nm, λ_{em} 450 nm); TIC detection mode; absorbance detection mode (254 nm); absorbance detection mode (375 nm).

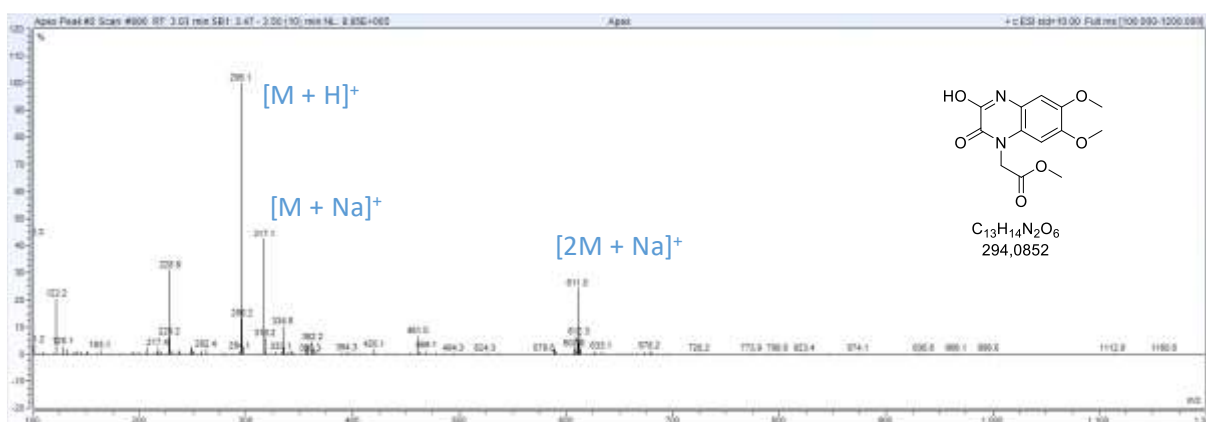


Figure S28 ESI mass spectrum associated to the peak at $t_{\text{R}}(\text{MS}) = 3.03$ min (plausible chemical structure).

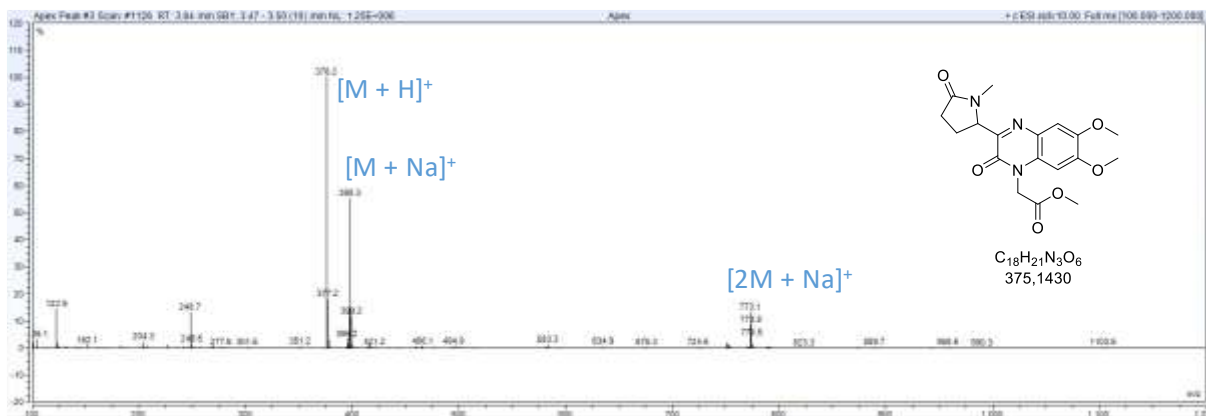


Figure S29 ESI mass spectrum associated to the peak at $t_{R(MS)} = 3.84$ min (plausible chemical structure).

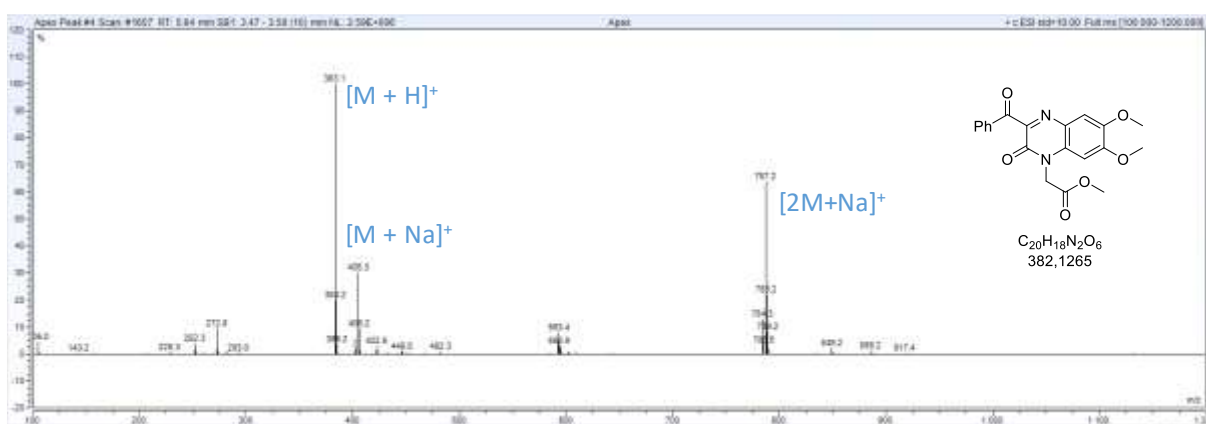


Figure S30 ESI mass spectrum associated to the peak at $t_{R(MS)} = 5.64$ min (structure confirmed with an authentic standard).

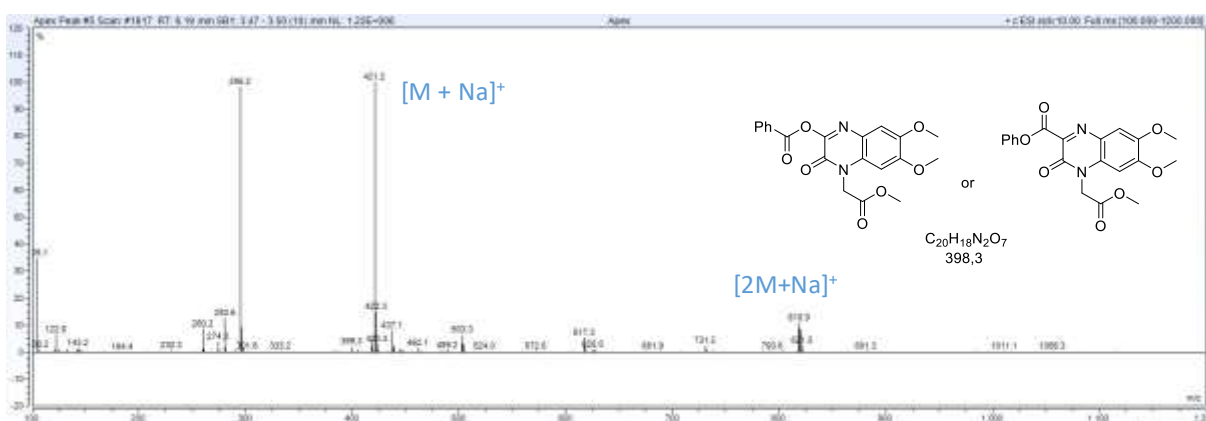


Figure S31 ESI mass spectrum associated to the peak at $t_{R(MS)} = 6.19$ min (plausible chemical structures).

C) Different mechanistic proposals for the formation of the fluorescent photoproduct **7**

Mechanism 1: The benzophenone-like mechanism (mechanism 1, Figure 32) involves the formation of a 1,2-diradical intermediate that subsequently traps the pyrrolidinone through a C-H insertion at the α -carbon of the amide group. However, this mechanism is unlikely because the corresponding product was not observed.

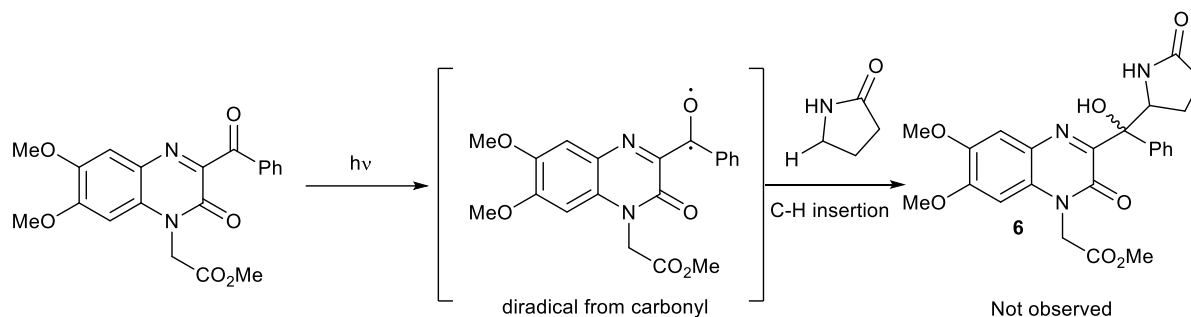


Figure S32 Mechanism 1: via the formation of diradical of carbonyl (benzophenone-like mechanism).

Mechanism 2: Although less exemplified than carbonyl derivatives, imines can be photochemically excited to deliver corresponding diradicals.⁶⁻⁹ In this context, a diradical of imine will be formed upon photoexcitation of **5**, which can then insert into the C-H bond of pyrrolidinone (mechanism 2, Figure 32). Then, homolysis of the ketone Intermediate I followed by the abstraction of the N-H proton by the benzoyl radical would form the corresponding product **7**.

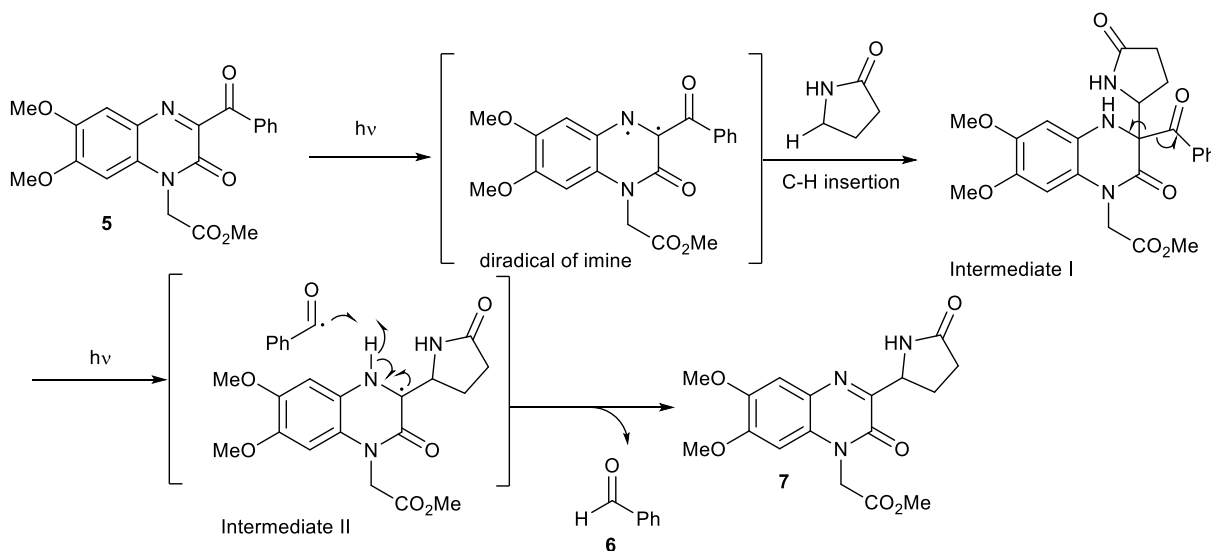


Figure S33 Mechanism 2: via the formation of diradical of imine.

Mechanism 3: The photochemical cleavage or homolysis of the ketone **5** generates two free radical intermediates (mechanism 3, Figure 34).¹⁰ These fragments will subsequently react with pyrrolidinone through a C-H insertion process to afford compound **7**.

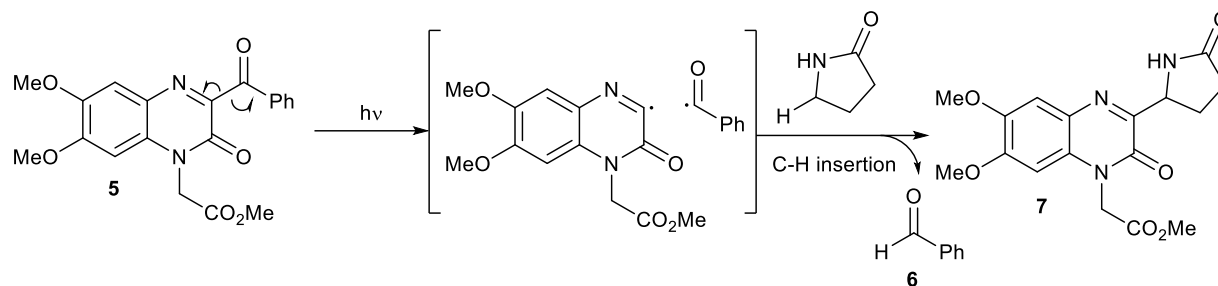
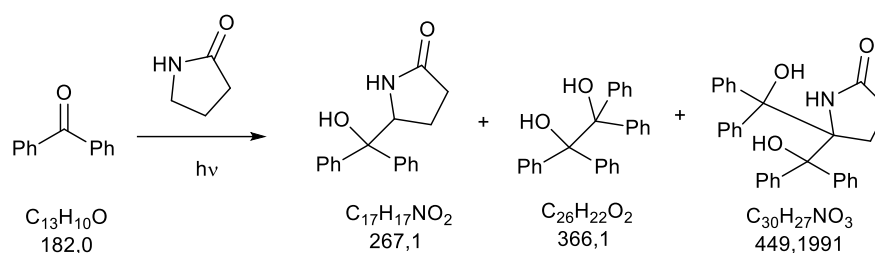


Figure 34: Mechanism 3: via Norrish type I photofragmentation.

D) Photoreaction between benzophenone and pyrrolidinone



Procedure: Benzophenone (1 mg, 5 μmol) in 2-pyrrolidinone (420 mg, 4.9 μmol , 900 equiv) was irradiated in a glass tube with a UV LED spotlight (λ_{max} 465 nm) for 10 min and 30 min. The reaction was monitored by analytical RP-HPLC-MS (Figures S35-40) or RP-HPLC (Figure S41).

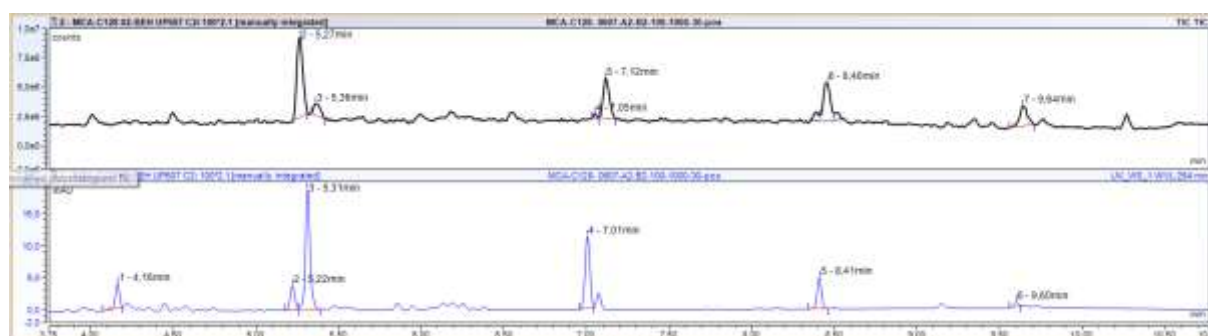


Figure S35 RP-HPLC-MS chromatograms of the photoreaction between benzophenone and pyrrolidinone after 10 min under LED irradiation (λ_{max} 365 nm), from top to bottom: TIC detection mode; absorbance detection mode (254 nm).

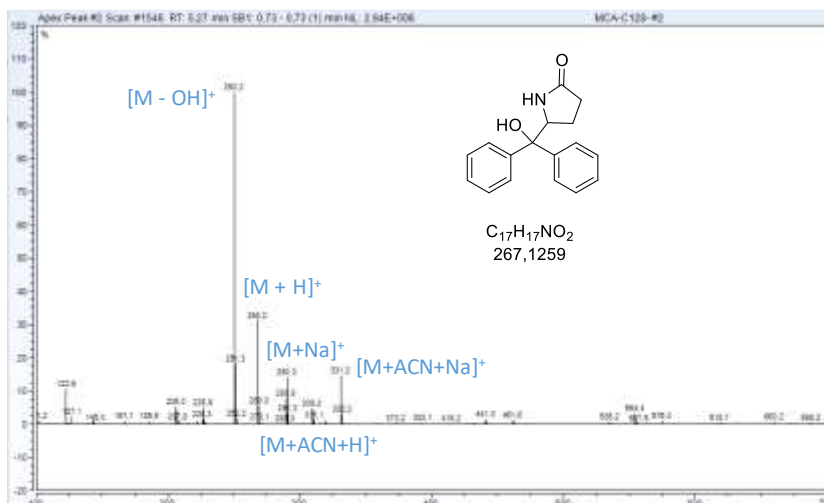


Figure S36 ESI mass spectrum associated to the peak at $t_{R(MS)} = 5.22$ min (plausible chemical structure).

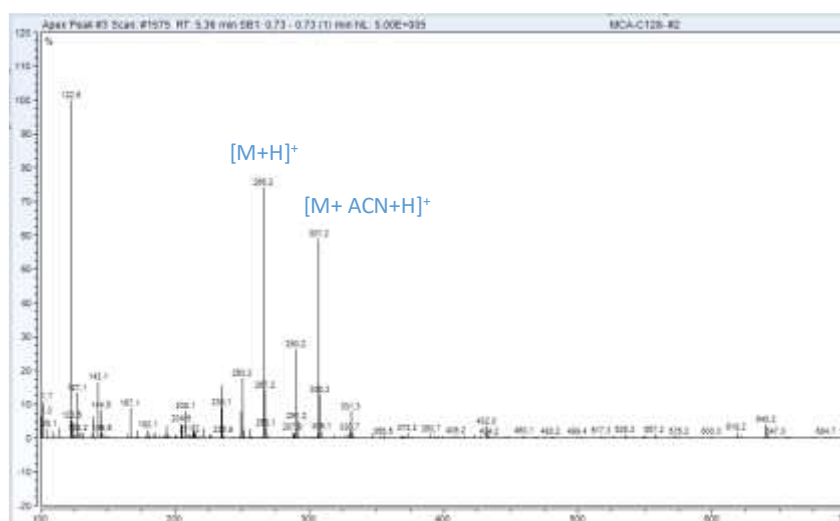


Figure S37 ESI mass spectrum associated to the peak at $t_{R(MS)} = 5.31$ min.

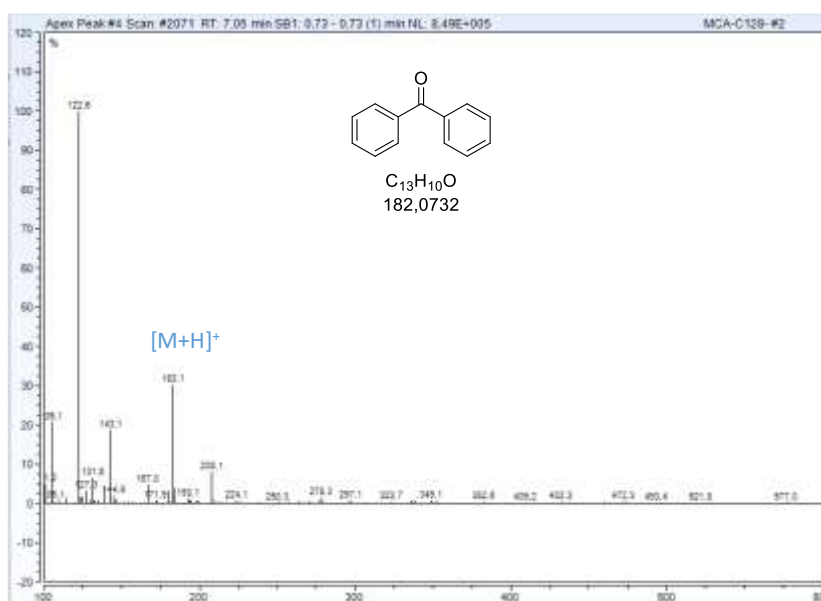


Figure S38 ESI mass spectrum associated to the peak at $t_{R(MS)} = 8.41$ min (chemical structure confirmed with an authentic standard).

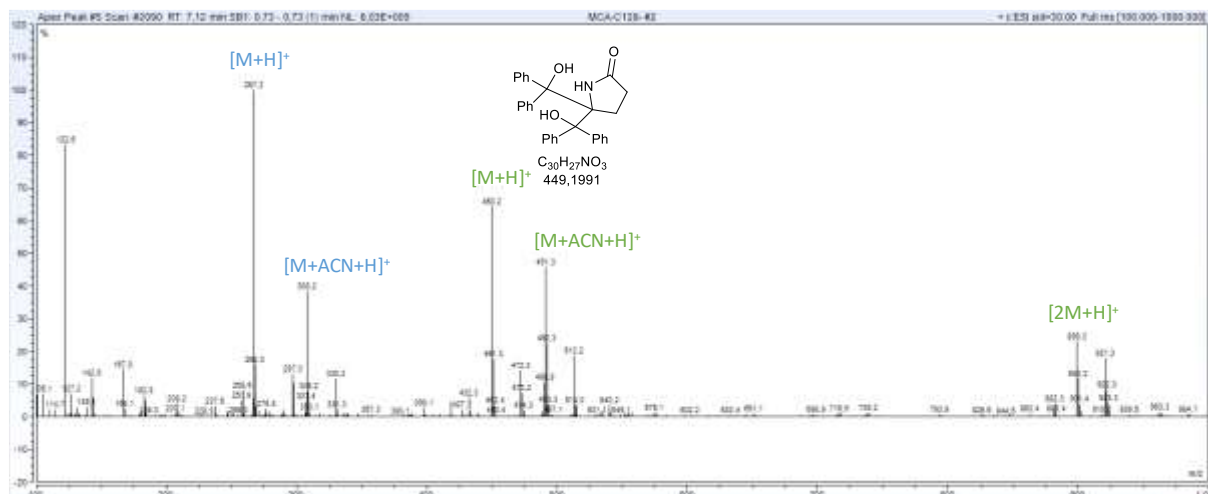


Figure S39 ESI mass spectrum associated to the peak at $t_{R(MS)} = 7.12$ min (plausible chemical structure).

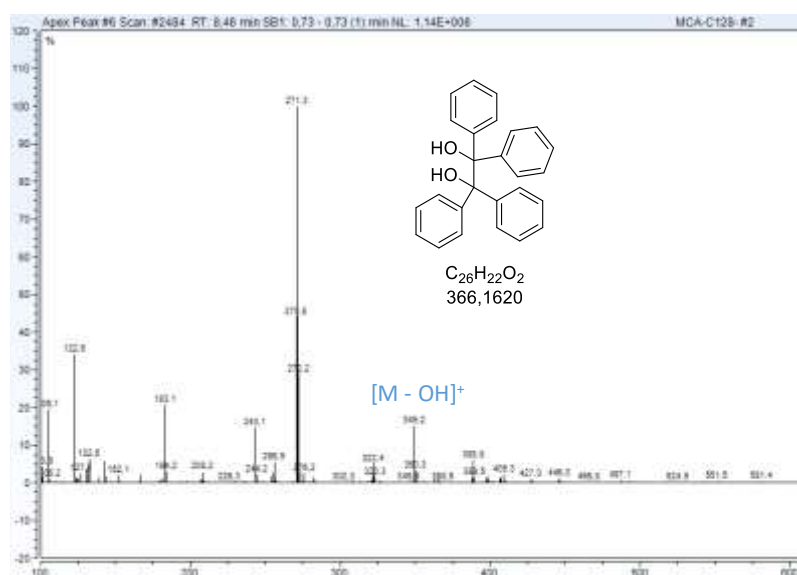


Figure S40 ESI mass spectrum associated to the peak at $t_{R(MS)} = 8.41$ min (chemical structure confirmed with an authentic standard).

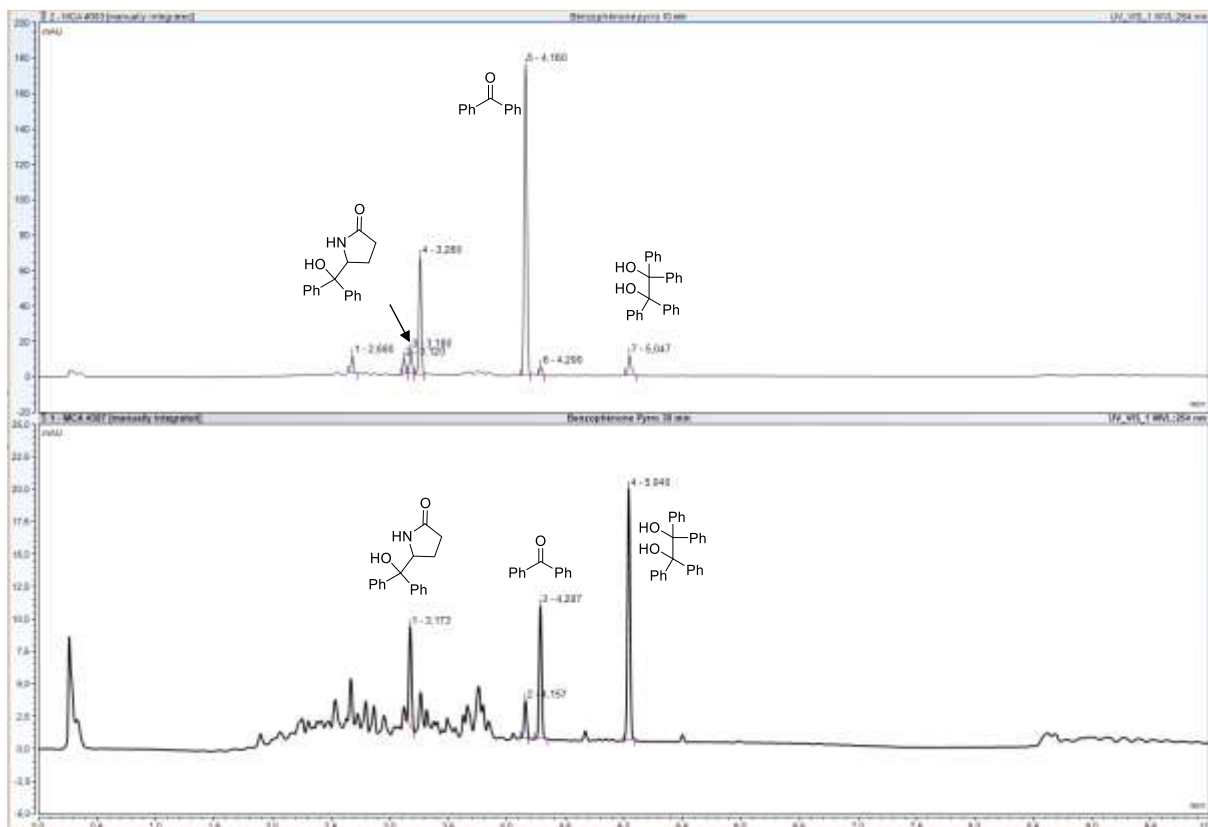


Figure S41 RP-HPLC chromatograms of the photoreaction between benzophenone and pyrrolidinone with UV detection (254 nm): from top to bottom: after 10 min ; after 30 min of UV LED irradiation (λ_{\max} 365 nm).

IX. References

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