Imaging of Mitochondria/Lysosome in Live cells and C. elegans

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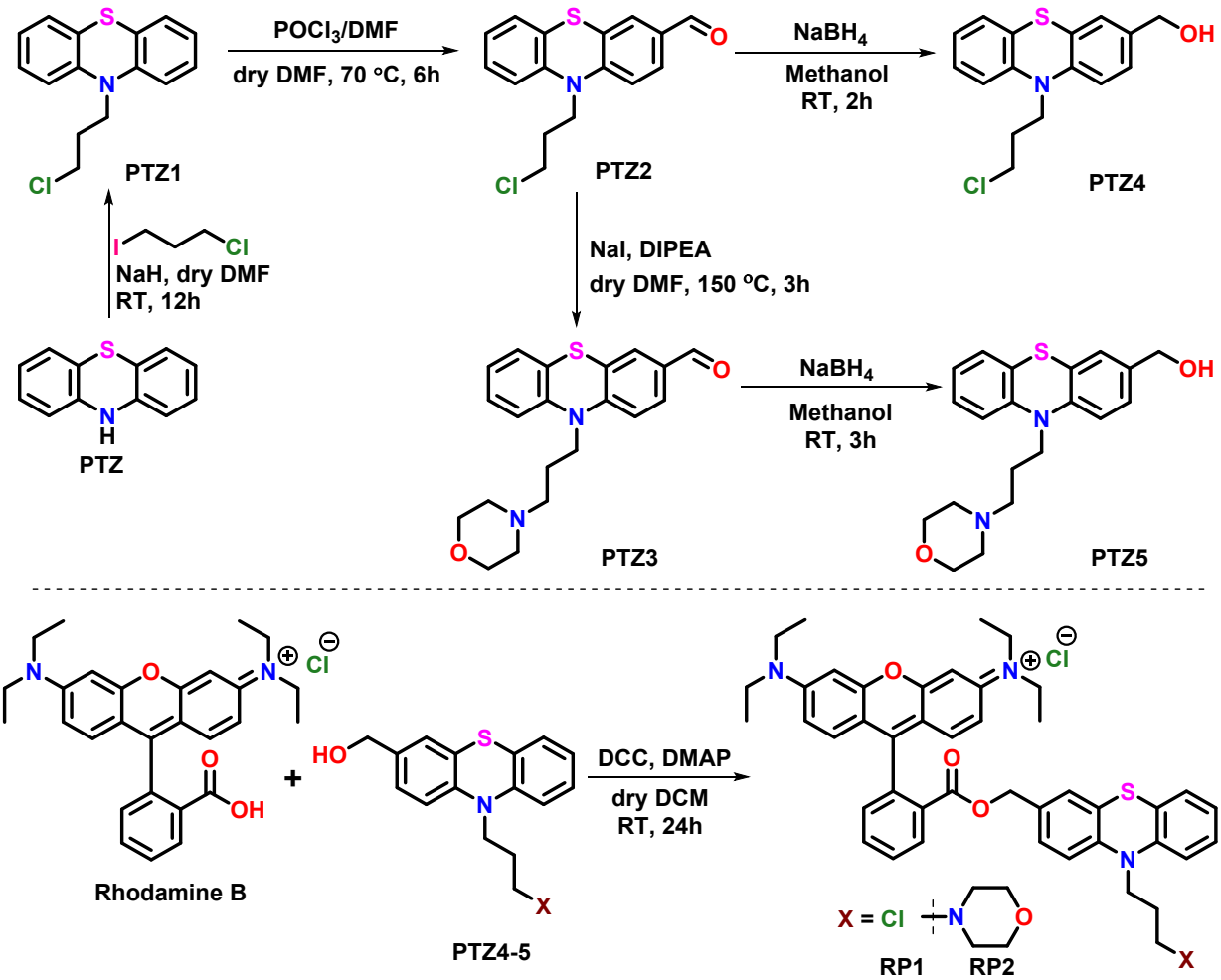
Video S1: The video showing (arrows) the appearance of circular structure (Lysosomes) after changes in the mitochondrial morphology: Compound used RP1.

Video S2: RP2 shows tracking of Lysosomes in real time inside cells. Lysosomes (in green color) show contact with mitochondria during its photo induced damage

1. Synthetic Procedure and Characterization Data:



Scheme S1: Synthetic routes to Rhodamine-Phenothiazine conjugates **RP1** and **RP2**.

Synthesis of PTZ1: In a round-bottom flask, 1-chloro-3-iodopropane was mixed with dry DMF and degassed with nitrogen. Then NaH was added to the mixture at 0 °C in an ice-bath. This was followed by adding a solution of phenothiazine (PTZ) in DMF slowly at room temperature, stirring the reaction mixture at ambient temperature for 12 hours. The progress of the reaction was monitored by TLC analysis at regular intervals. Then the reaction mixture was neutralized with saturated $NaHCO_3$ solution. The mixture was washed with ethyl acetate and water twice, an organic layer was collected over anhydrous Na_2SO_3, and dried under a rotary evaporator. The crude mixture was purified using silica-gel column chromatography in hexane/ethyl acetate (99:1) eluent. An oily product was obtained, which formed a white crystalline solid upon freezing in a refrigerator. The obtained product was characterized as desired by NMR and mass analysis. Yield = 82%, white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 7.19-7.15 (m,

4H), 6.95-6.90 (m, 4H), 4.09 (t, *J* = 6.5 Hz, 2H), 3.67 (t, *J* = 6.1 Hz, 2H), 2.24 (p, *J* = 6.4 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm) 145.09, 127.65, 127.31, 125.74, 122.74, 115.58, 43.97, 42.44, 29.70. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{15}H_{15}ClNS$ $[M]^+$: 275.0535; Found: 275.0538; error: 1.09 ppm.

Synthesis of PTZ2: In a round-bottom flask, dry DMF was added dropwise to a $POCl_3$ solution under an argon atmosphere using a dropping funnel at room temperature. This mixture was stirred for 40 minutes at ambient temperature, resulting in a reddish solution. To this mixture, a solution of **PTZ1** in dry DMF was added dropwise to form a scarlet suspension. Then, the reaction mixture was stirred at 70 °C for 6 hours. The mixture was then treated with sodium hydroxide solution, washed with ethyl acetate and water twice, the organic layer was collected over anhydrous Na_2SO_4 and dried under a rotary evaporator. The crude compound was purified by silica-gel column chromatography in hexane/ethyl acetate (90:10) eluent. Yield = 64%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 9.82 (s, 1H), 7.68 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.63 (d, *J* = 1.8 Hz, 1H), 7.22-7.14 (m, 2H), 7.03-6.92 (m, 3H), 4.16 (t, *J* = 6.6 Hz, 2H), 3.68 (t, *J* = 6.0 Hz, 2H), 2.26 (p, *J* = 6.4 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm) 190.03, 150.70, 143.23, 131.45, 130.11, 128.62, 127.84, 127.71, 125.99, 124.65, 123.94, 116.15, 115.12, 44.44, 42.07, 29.44. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{16}H_{15}ClNOS$ $[M+H]^+$: 304.0563; Found: 304.0562; error: 0.33 ppm.

Synthesis of PTZ3: In a round-bottom flask, **PTZ2** was dissolved in dry DMF, and diisopropylethylamine (DIPEA) was added under an argon atmosphere. Then, morpholine was added to the solution, followed by sodium iodide. The mixture was stirred under an argon atmosphere at 150 ℃ for 3 hours. After cooling to room temperature, the mixture was poured into water and washed twice with ethyl acetate. The combined organic layers were washed with brine solution. The organic layer was collected over anhydrous sodium sulfate and concentrated under a rotary evaporator. The crude product was purified by silica-gel column chromatography using ethyl acetate/methanol (95:5). The product was obtained as desired and characterized. Yield 76%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 9.80 (s, 1H), 7.64 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.19-7.14 (m, 1H), 7.12 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.00-6.91 (m, 2H), 4.01 (t, *J* = 6.9 Hz, 2H), 3.68-3.59 (t, 4H), 2.47 (t, *J* = 6.9 Hz, 2H), 2.41 (broad s, 4H), 1.99-1.91 (q, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm) 190.06, 150.80, 143.37, 131.13, 130.05, 128.49, 127.63, 127.59, 125.23, 124.02, 123.69, 116.11, 114.98, 67.00, 55.61, 53.79, 45.58, 23.91. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{20}H_{23}N_2O_2S$ $[M+H]^+$: 355.1480; Found: 355.1480; error: 0.00 ppm.

Synthesis of PTZ4: In a round-bottom flask, sodium borohydride was added to a stirred solution of **PTZ2** in methanol at 0 °C in an ice-bath. The reaction mixture was allowed to warm to ambient temperature and stirred for another 2 hours. Then, the mixture was concentrated under reduced pressure, and the residue was diluted with ethyl acetate. This mixture was washed with brine solution and water, the organic layer was collected over anhydrous sodium sulfate and evaporated under a rotary evaporator to dryness. An oily product obtained as desired and characterized. Yield 93%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 7.22-7.12 (m, 4H), 6.91 (m, 3H), 4.58 (s, 2H), 4.08 (t, *J* = 6.4 Hz, 21H), 3.67 (t, *J* = 6.1 Hz, 2H), 2.23 (p, *J* = 6.3 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm) 144.95, 144.62, 135.42, 127.67, 127.38, 126.55, 126.33, 126.04, 125.45, 122.81, 115.57, 115.55, 64.68, 43.96, 42.40, 29.62. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{16}H_{17}ClNOS$ $[M]^+$: 305.0641; Found: 305.0677; error: 11.8 ppm.

Synthesis of PTZ5: In a round-bottom flask, sodium borohydride was added to a stirred solution of **PTZ3** in methanol at 0 °C in an ice bath. The reaction mixture was allowed to warm to ambient temperature and stirred for another 3 hours. Then, the mixture was concentrated under reduced pressure, and the residue was diluted with ethyl acetate. This mixture was washed with brine solution and water, the organic layer was collected over anhydrous sodium sulfate and evaporated under a rotary evaporator to dryness. An oily product obtained as desired and characterized. Yield 90%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 7.10-7.04 (m, 4H), 6.87-6.78 (m, 3H), 4.50 (s, 2H), 3.87 (t, *J* = 6.8 Hz, 2H), 3.61-3.58 (m, 4H), 2.41 (t, *J* = 7.0 Hz, 2H), 2.35 (broad s, 4H), 1.93-1.83 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm) 144.06, 143.69, 134.11, 126.46, 126.26, 125.37, 125.22, 124.42, 123.83, 121.50, 114.48, 114.45, 65.86, 63.65, 54.98, 52.71, 44.13, 22.90. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{20}H_{25}N_2O_2S$ $[M+H]^+$: 357.1637; Found: 357.1640; error: 0.84 ppm.

Synthesis of RP1: In a nitrogen-flushed round-bottom flask, Rhodamine B was dissolved in dry DCM. This was followed by the addition of N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), and stirred the mixture for 30 minutes at ambient temperature. Then, a solution of **PTZ5** in dry DCM was added to the above mixture, and stirred the resultant mixture at ambient temperature for 24 hours under nitrogen atmosphere. The progress of the reaction was monitored by TLC analysis. After 24 hours the rhodamine was not consumed completely, therefore the solvent was evaporated, and washed with DCM ad water twice. The organic layer was collected over anhydrous sodium sulfate, and dried. The crude compound was subjected to silica-gel column chromatography in DCM/methanol (97:3)

eluent. The desired product was obtained as purple solid. Yield 38%, Melting Point: 123 °C. **^{1}H NMR** (500 MHz, DMSO-D_6) δ (ppm) 8.28 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.90 (td, *J* = 7.5, 1.3 Hz, 1H), 7.84 (td, *J* = 7.7, 1.3 Hz, 1H), 7.47 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.31-7.25 (m, 1H), 7.23 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.09-7.01 (m, 3H), 6.97 (d, *J* = 9.5 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.78 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.69 (d, *J* = 2.3 Hz, 2H), 6.67 (d, *J* = 1.9 Hz, 1H), 4.84 (s, 2H), 4.05 (t, *J* = 6.7 Hz, 2H), 3.78 (t, *J* = 6.2 Hz, 2H), 3.62 (m, 8H), 2.18-2.10 (q, 2H), 1.22 (t, *J* = 7.0 Hz, 12H). **^{13}C NMR** (126 MHz, DMSO) δ (ppm) 165.15, 157.78, 157.31, 155.43, 144.97, 144.68, 133.68, 133.40, 131.47, 131.28, 130.97, 130.88, 129.80, 129.26, 128.32, 128.29, 127.70, 127.42, 124.50, 124.11, 123.52, 116.51, 115.83, 114.98, 113.29, 96.17, 66.73, 45.77, 44.07, 43.18, 29.66. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{44}H_{45}N_3O_3SCl$ $[M]^+$: 730.2865; Found: 730.2872; error: 0.96 ppm.

Synthesis of RP2: In a nitrogen flushed round-bottom flask, Rhodamine B was dissolved in dry DCM. This was followed by the addition of N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), and stirred the mixture for 30 minutes at ambient temperature. Then, a solution of **PTZ5** (phenothiazine with morpholine) in dry DCM was added to the above mixture, and stirred the resultant mixture at ambient temperature for 24 hours under nitrogen atmosphere. The progress of the reaction was monitored by TLC analysis. After 24 hours the rhodamine was not consumed completely, therefore the solvent was evaporated, and washed with DCM ad water twice. The organic layer was collected over anhydrous sodium sulfate, and dried. The crude compound was subjected to silica-gel column chromatography in DCM/methanol (94:6) eluent. The desired product obtained as purple solid. Yield 31%, Melting Point 138 °C. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 8.30 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.80 (td, *J* = 7.6, 1.3 Hz, 1H), 7.72 (td, *J* = 7.8, 1.2 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 7.21-7.17 (m, 1H), 7.12 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.04 (d, *J* = 9.5 Hz, 2H), 6.95 (m, 2H), 6.89-6.84 (m, 3H), 6.78 (d, *J* = 8.3 Hz, 1H), 6.72 (dd, *J* = 4.8, 2.2 Hz, 3H), 4.86 (s, 2H), 3.95 (t, *J* = 6.8 Hz, 2H), 3.63 (m, 12H), 2.48 (t, *J* = 7.0 Hz, 2H), 2.41 (broad s, 4H), 1.94 (q, 2H), 1.33 (t, *J* = 7.1 Hz, 12H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm) 163.94, 157.29, 156.53, 154.44, 144.36, 143.62, 132.37, 132.07, 130.50, 130.16, 129.36, 129.15, 128.86, 127.37, 126.98, 126.62, 126.23, 124.10, 123.27, 121.84, 114.82, 114.45, 113.16, 112.46, 95.43, 65.85, 65.51, 54.69, 52.47, 45.13, 44.27, 28.68, 11.71. **HRMS** (ESI-ToF) *m/z*: Calculated for $C_{48}H_{53}N_4O_4S$ $[M]^+$: 781.3783; Found: 781.3790; error: 0.96 ppm.

Colocalization of RP1 in HeLa cells:

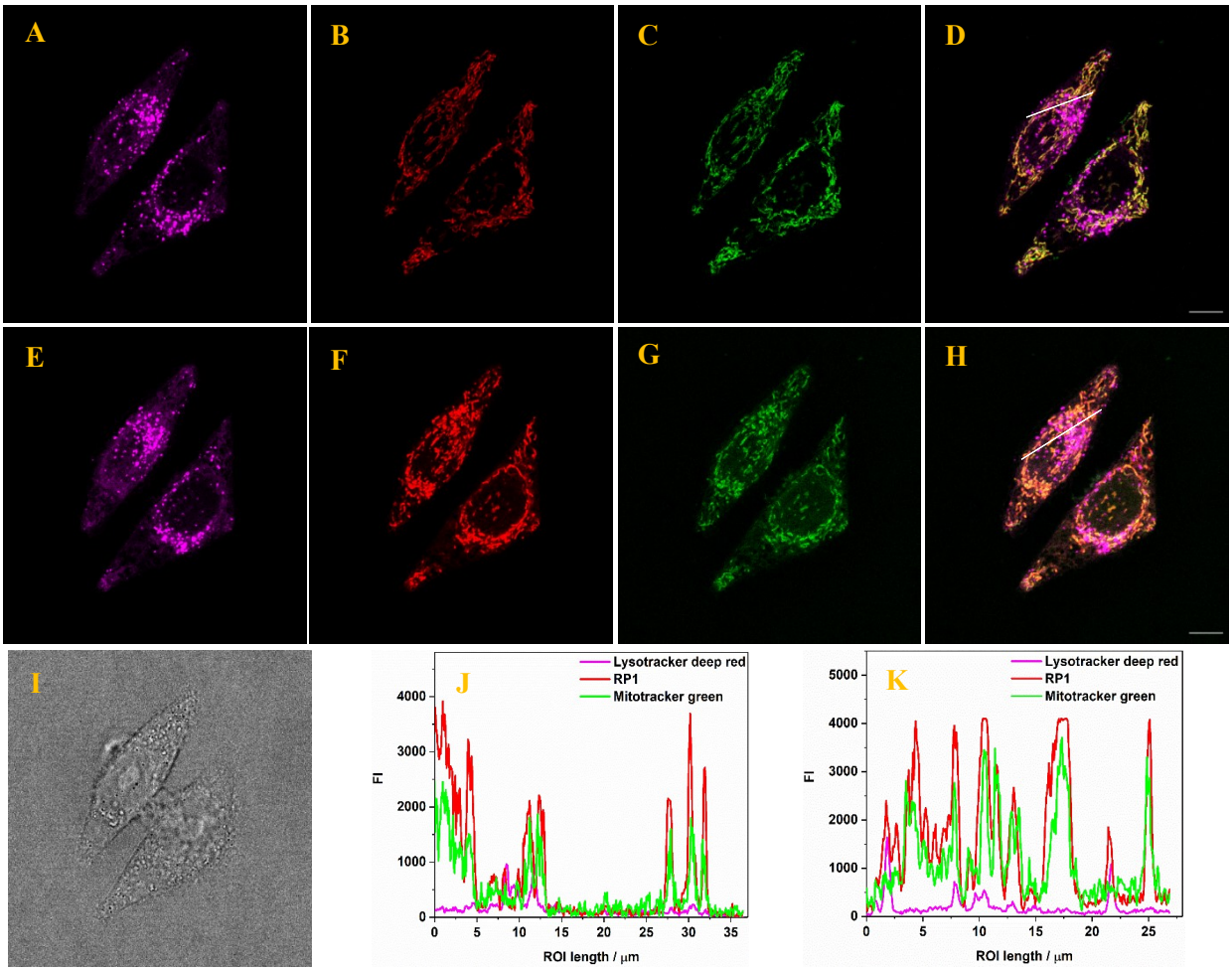


Figure S1: Colocalization experiment with **RP1** in HeLa cells with LysoTracker™ Deep Red and MitoTracker™ Green FM. The upper panel (A-D) are the initial images and middle panel (E-H) represents cells exposed to laser. The magenta channel (A & E) represents cells stained LysoTracker™ Deep Red, the red channel (B & F) are cells stained with **RP1**, the green channel (C & G) are MitoTracker™ Green FM stained cells and D & F represent the merged images of the three channels. I is Bright field image. J and K are the fluorescence intensity of the region of interest as shown in merged images of D and H, respectively. Scale bar 10 μm.

Colocalization of RP2 in HeLa cells:

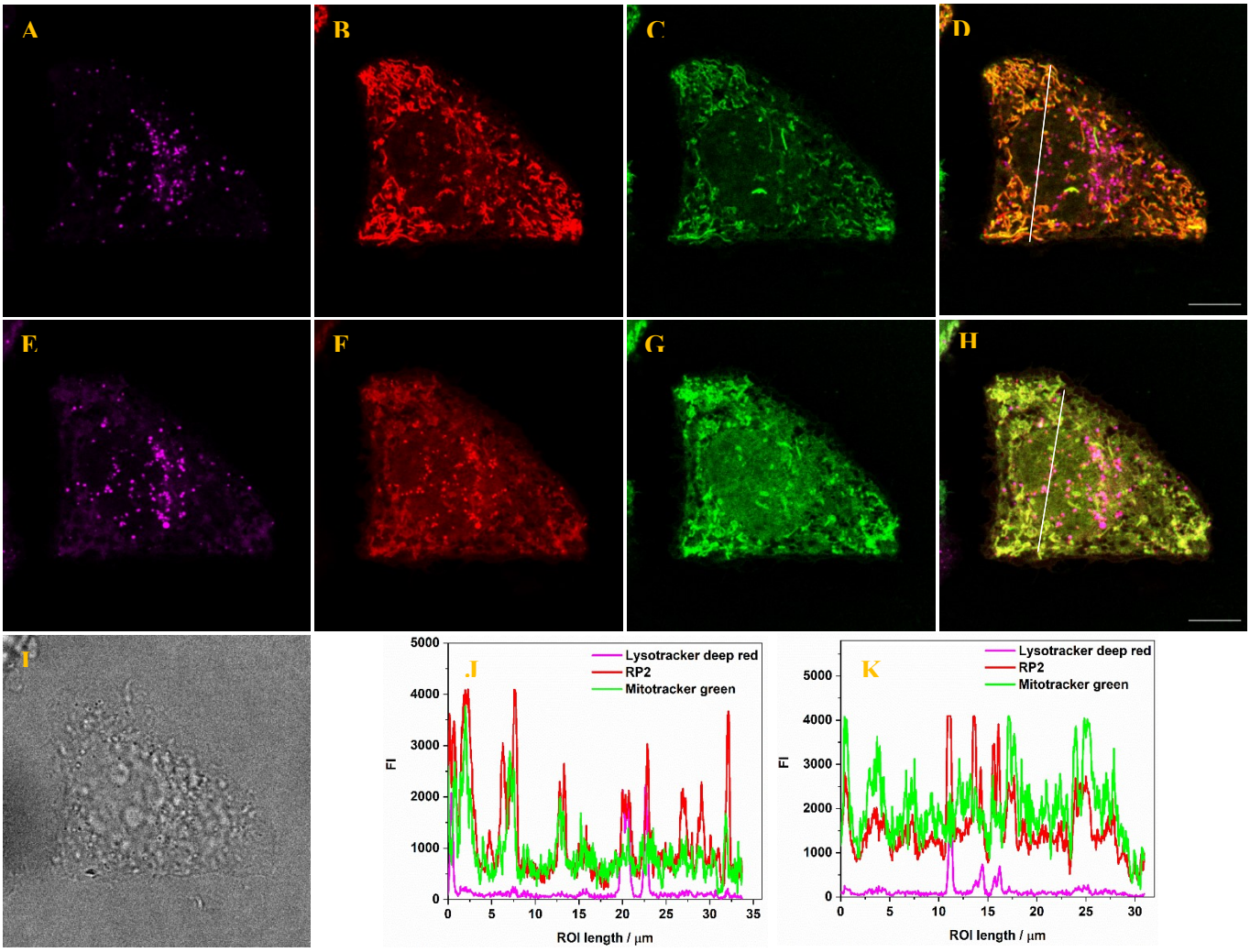


Figure S2: Colocalization experiment with **RP2** in HeLa cells with LysoTracker™ Deep Red and MitoTracker™ Green FM. The upper panel (A-D) are the initial images and middle panel (E-H) represents cells exposed to laser. The magenta channel (A & E) represents cells stained LysoTracker™ Deep Red, the red channel (B & F) are cells stained with **RP2**, the green channel (C & G) are MitoTracker™ Green FM stained cells and D & F represent the merged images of the three channels. I is Bright field image. J and K are the fluorescence intensity of the region of interest as shown in merged images of D and H respectively. Scale bar 10 µm

COS-7 cells incubated for 24 h with **RP1** and **RP2:**

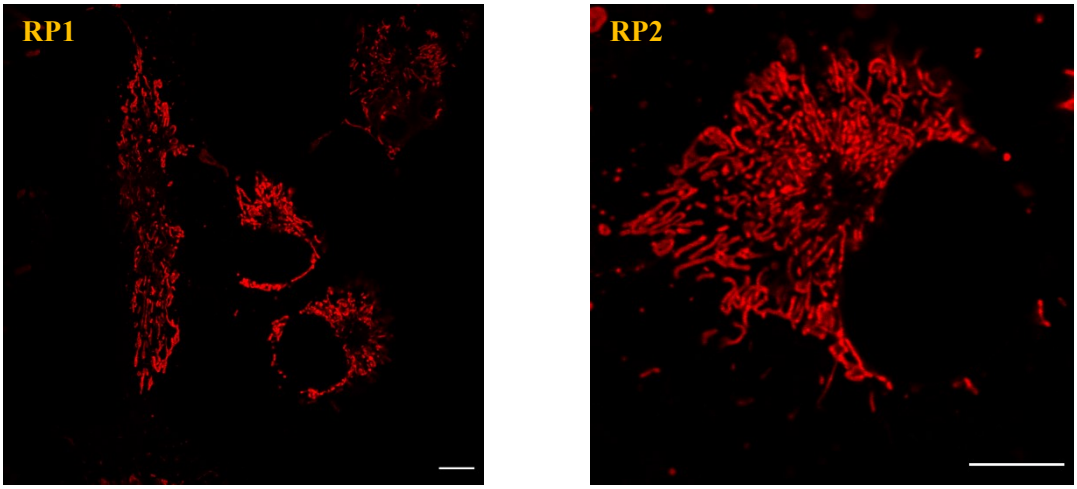


Figure S3: Confocal images of COS-7 cells after incubation of **RP1** and **RP2** for 24 h. Scale bar is 10 μm.

MTT data for RP1 and RP2:

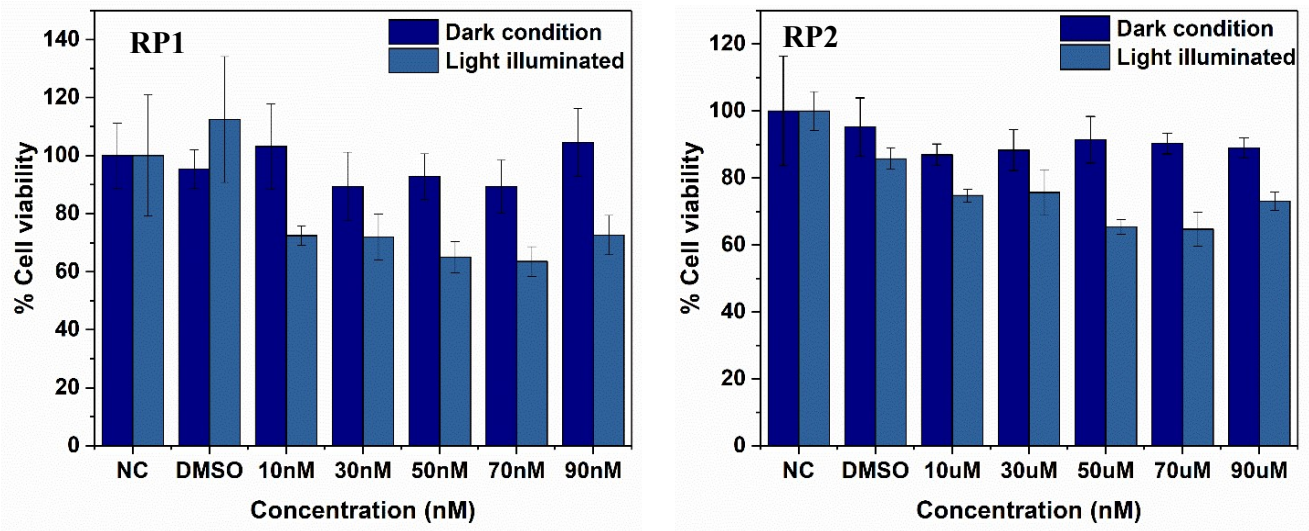


Figure S4: Bar graph representing MTT assay data for **RP1** and **RP2** in the dark and with light illumination in COS-7 cells. NC stand for negative control in the graph.

ROS detection in live-cells with RP2:

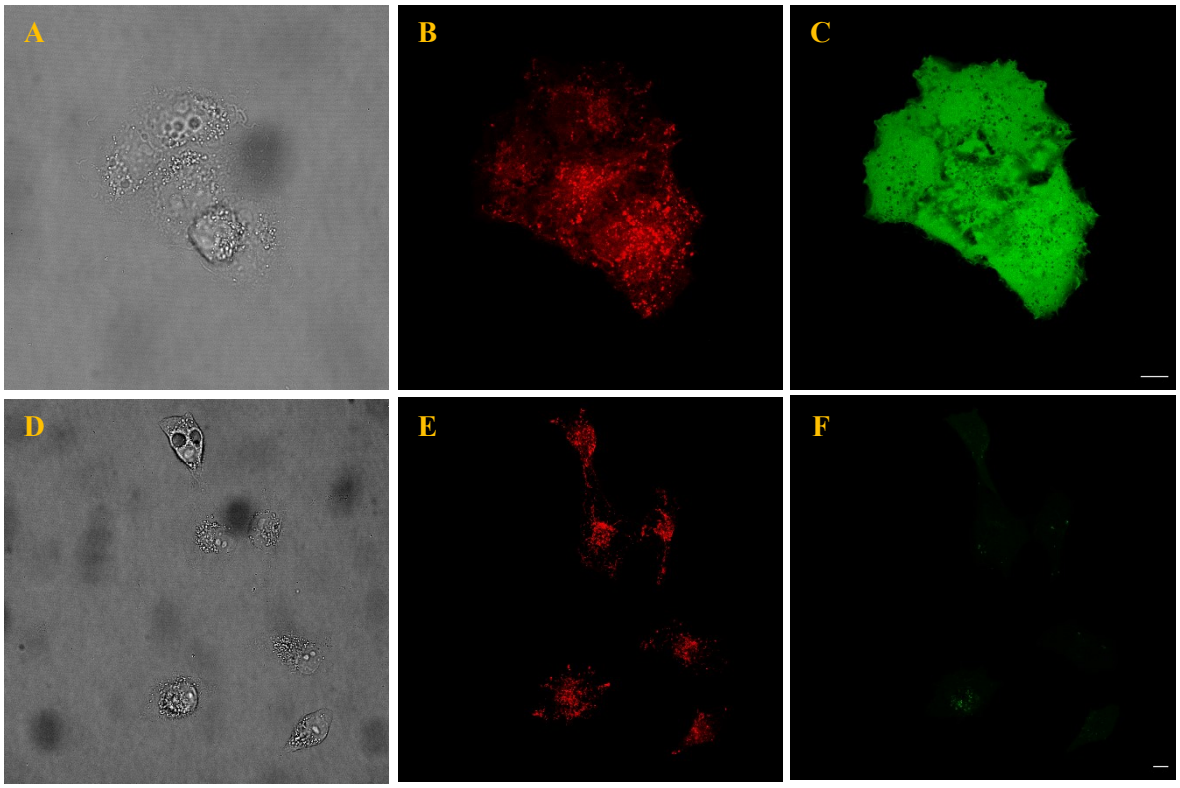


Figure S5: The ROS detection in COS-7 cells using DCFH-DA. The laser treatment panel A-C are treated with **RP2** and DCFH-DA then exposed to laser, showing strong emission in green channel. The Control panel D-F are treated with **RP2** and DCFH but no laser exposure, there was no signal in green channel. A & D are bright field images, B & E are cells excited at **RP2** dye wavelength (561 nm laser) and C & F are cells excited at 488 nm laser corresponding to DCF excitation. Scale bar is 10 μm.

Singlet Oxygen Studies for RP2

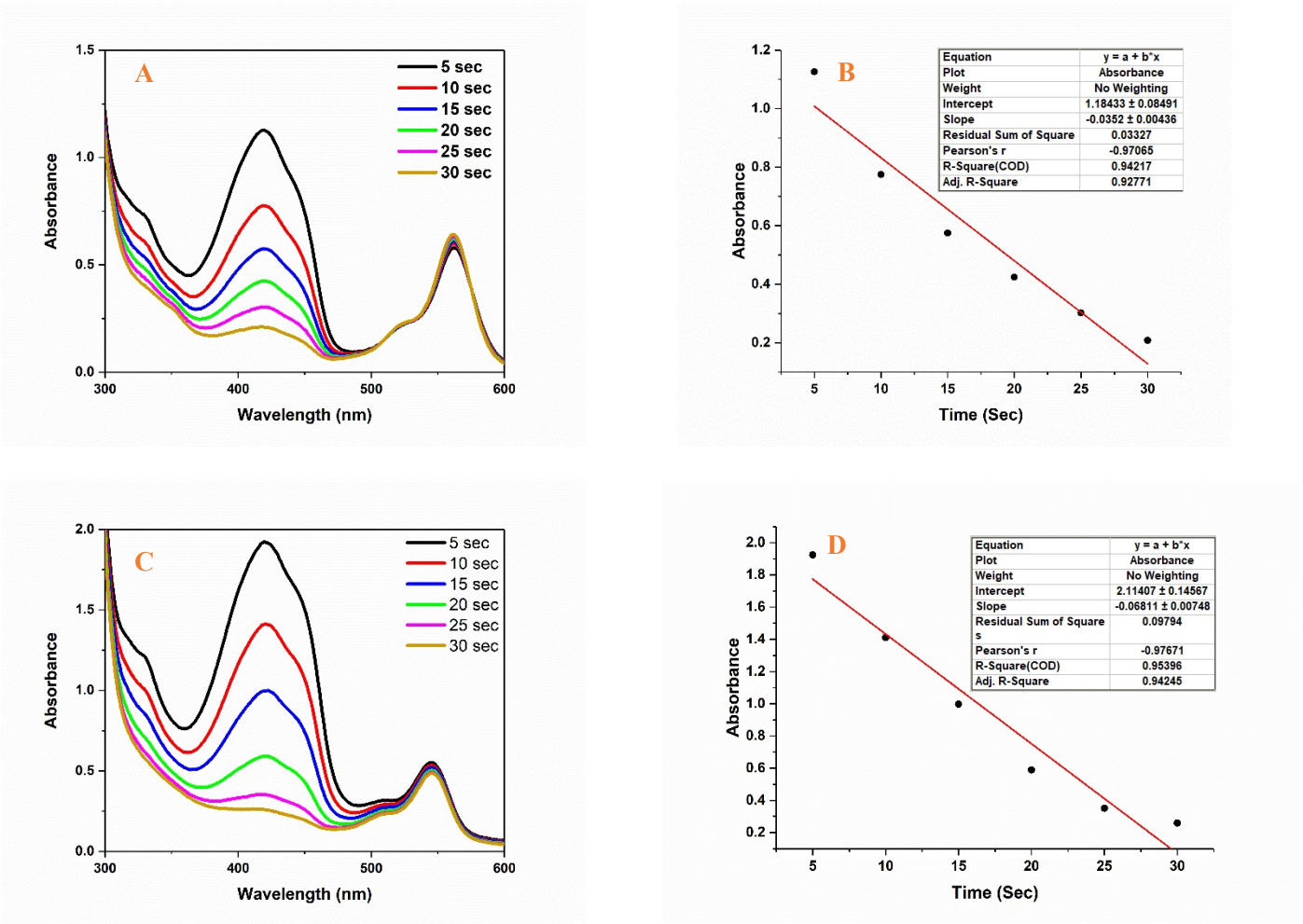


Figure S6: UV-vis spectral changes for **RP2** (A & B) and Rose Bengal (C & D) in water upon photoirradiation with the LED white lamp (3 W) to monitor the gradual decrease in absorption of DPBF (measured at 420 nm) with time.

NMR and Mass Spectra:

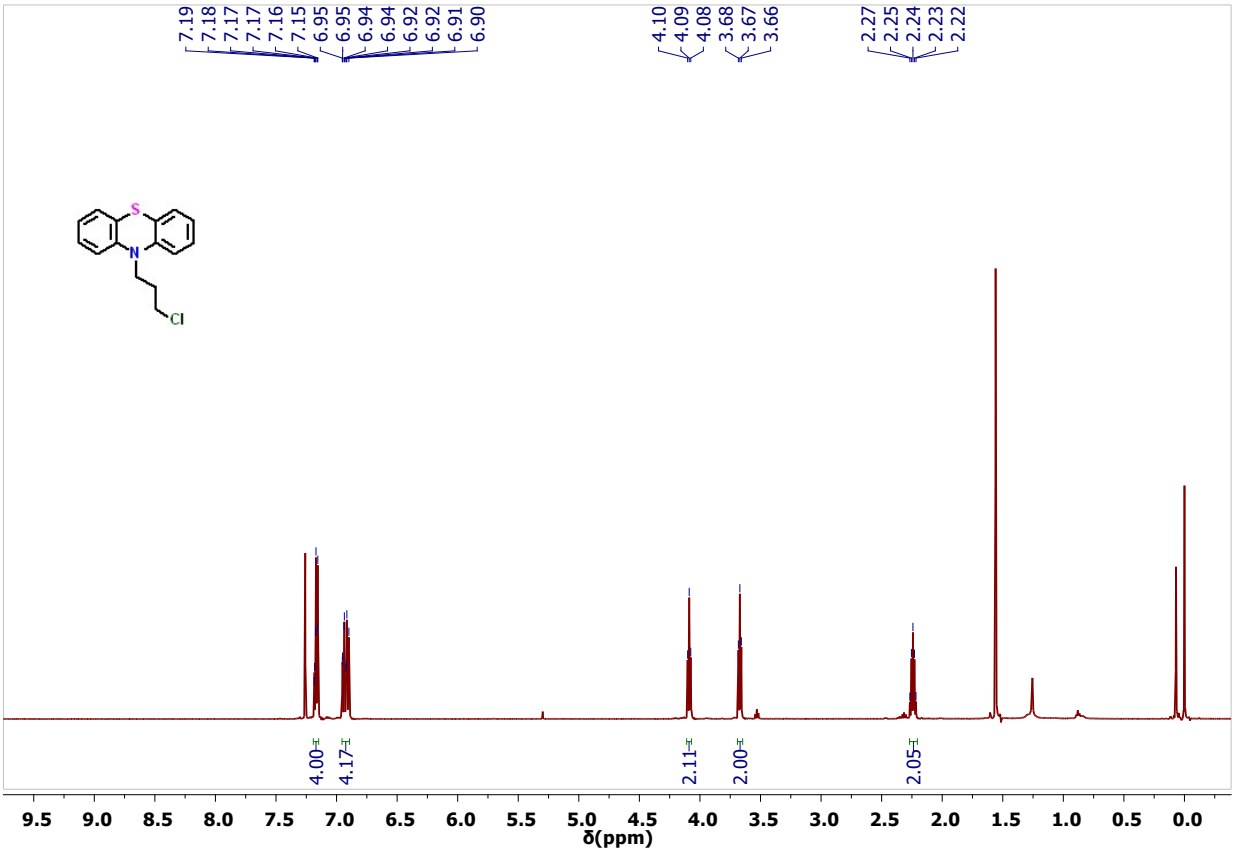


Figure S7: ^{1}H NMR spectra of **PTZ1** in $CDCl_3$.

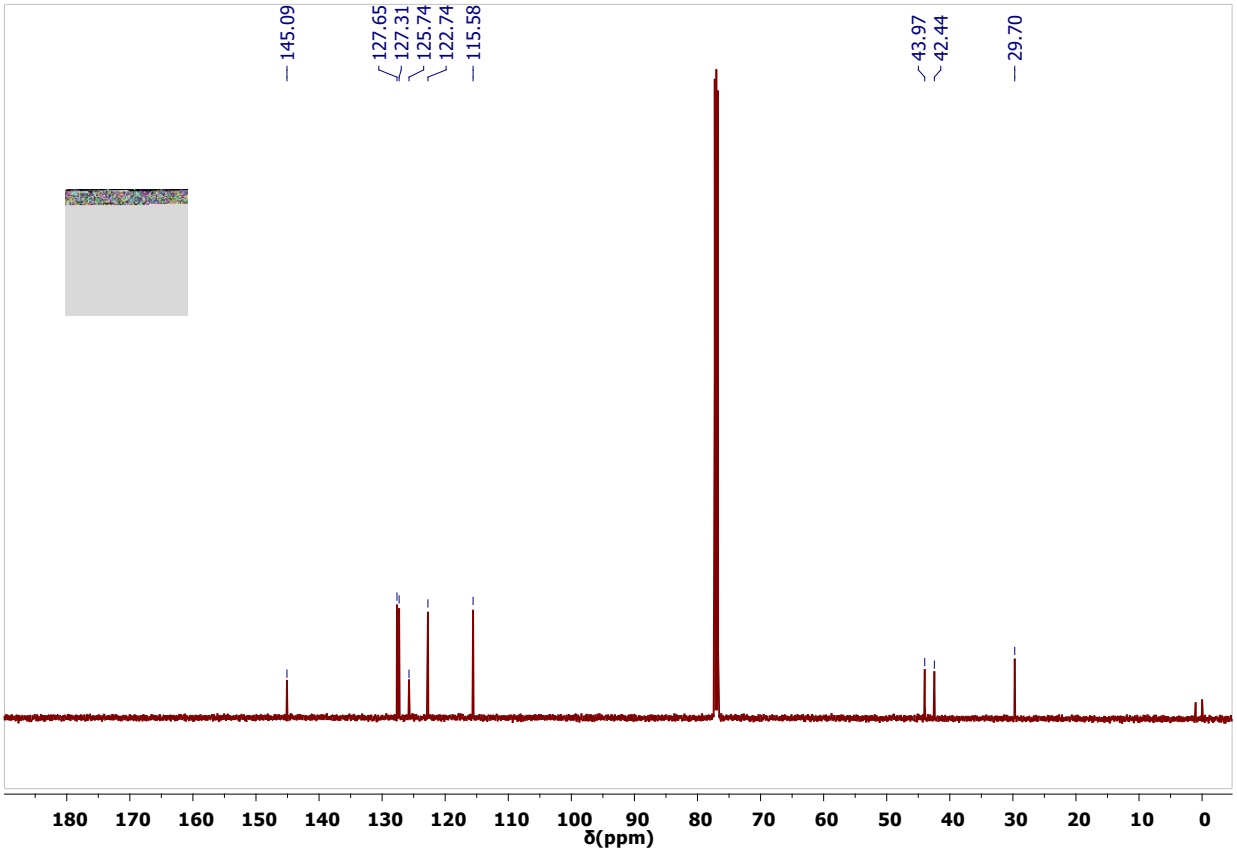


Figure S8: ^{13}C NMR spectra of **PTZ1** in $CDCl_3$.

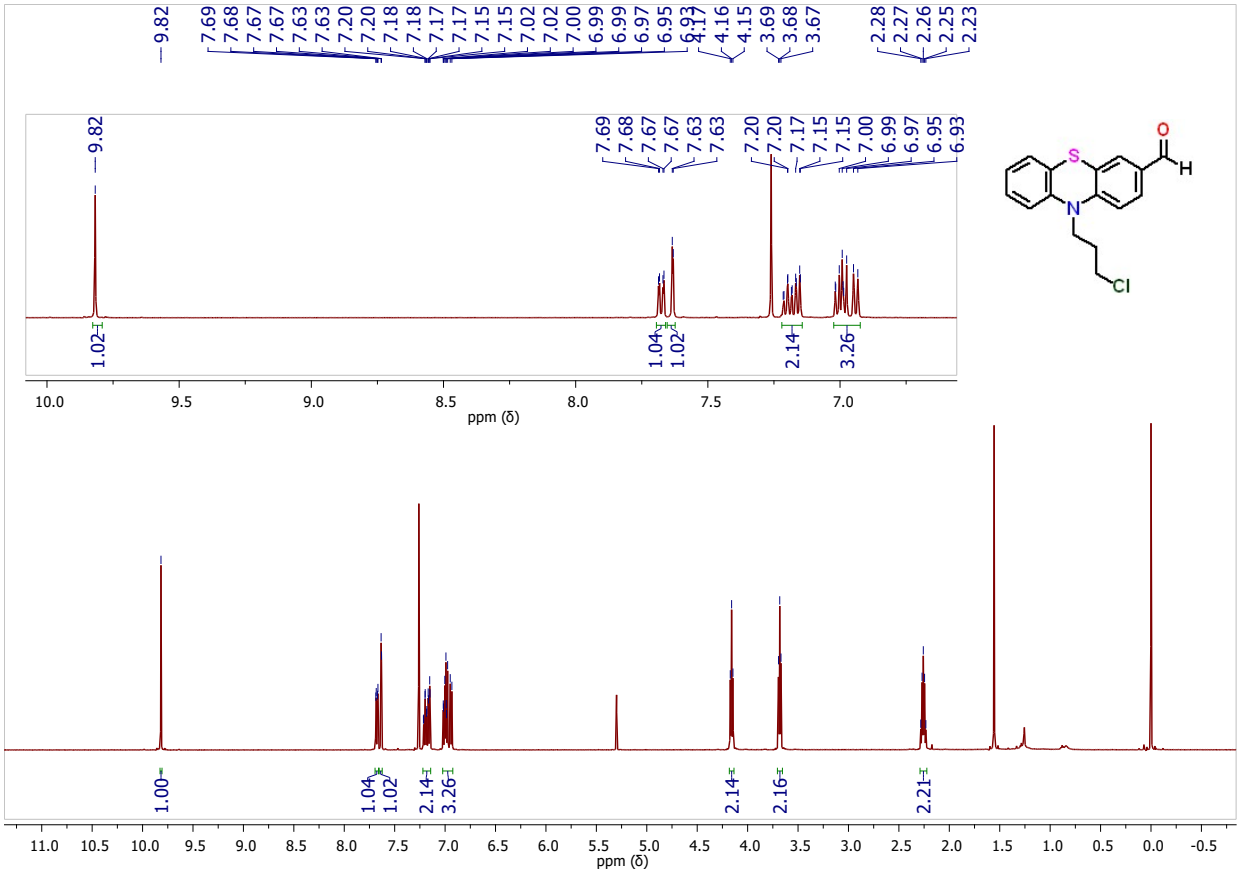


Figure S9: ^{1}H NMR spectra of **PTZ2** in $CDCl_3$.

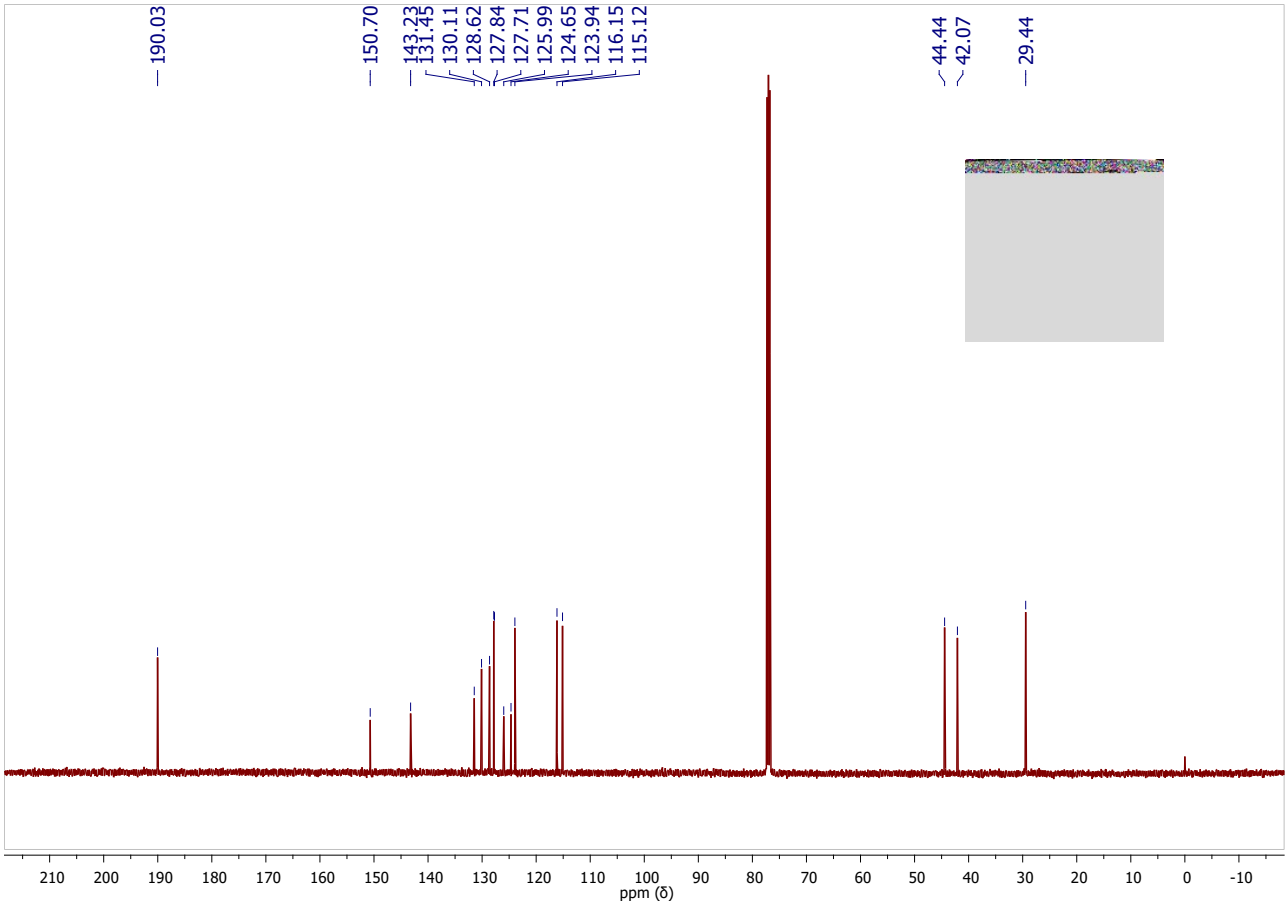


Figure S10: ^{13}C NMR spectra of **PTZ2** in $CDCl_3$.

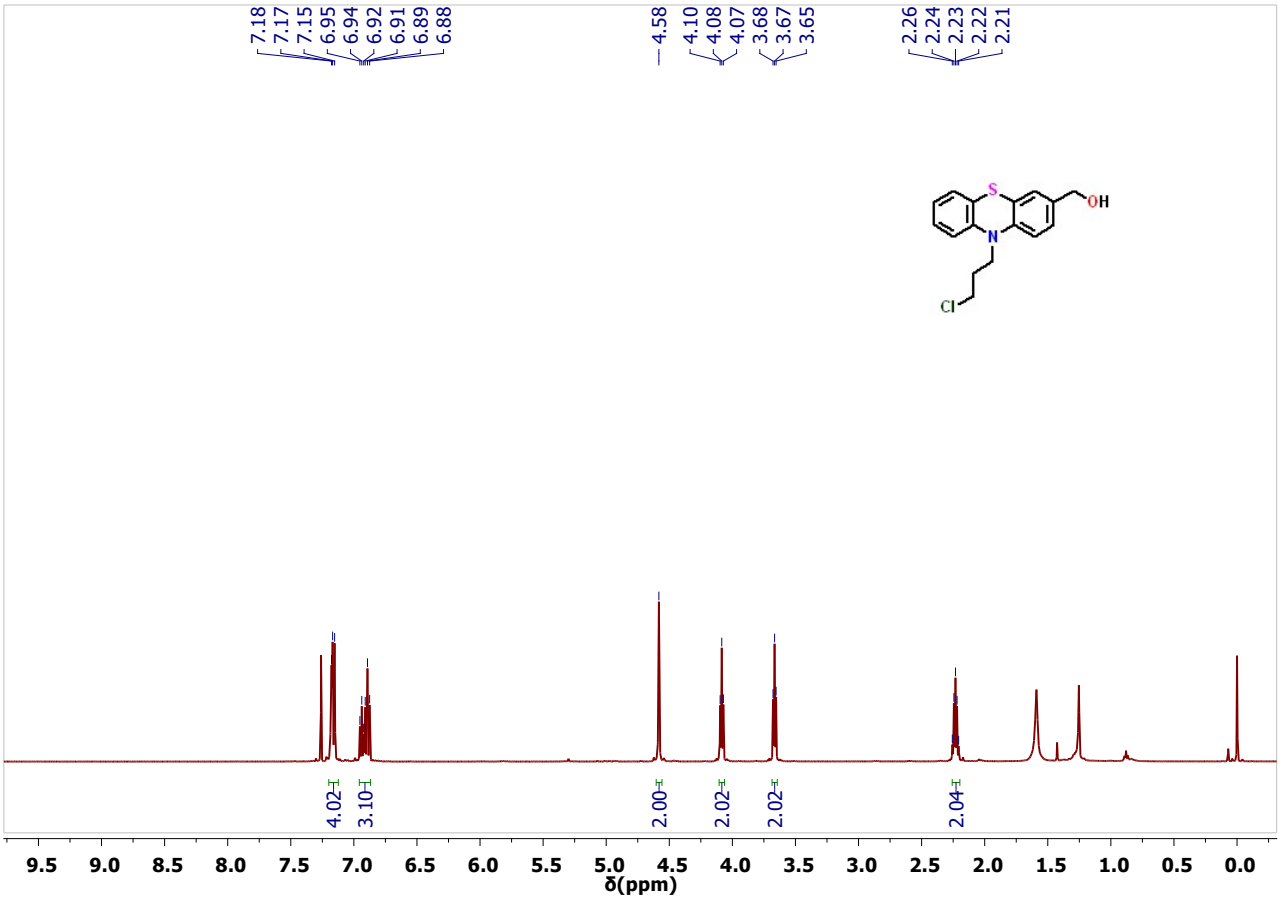


Figure S11: ^{1}H NMR spectra of **PTZ4** in $CDCl_3$.

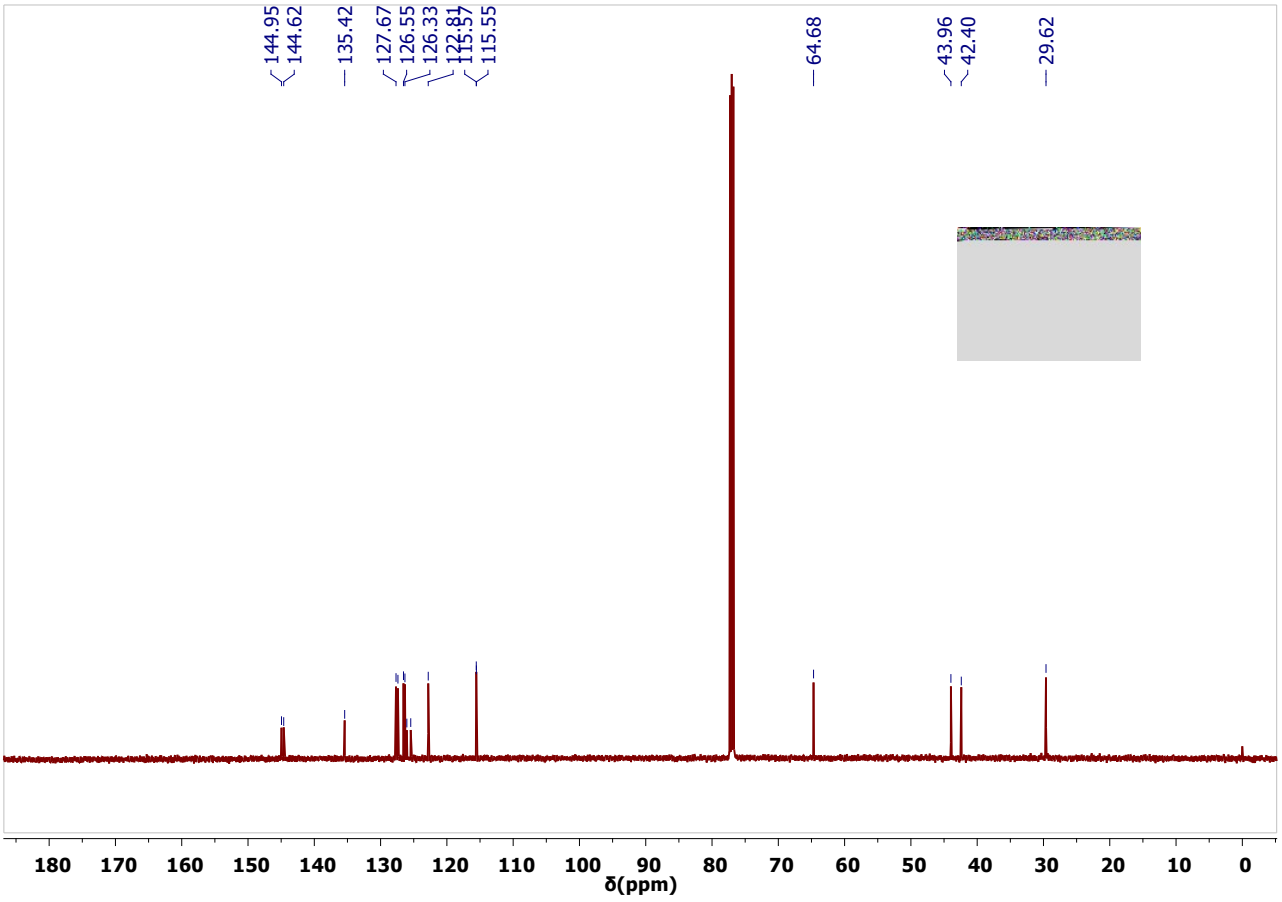


Figure S12: ^{13}C NMR spectra of **PTZ4** in $CDCl_3$.

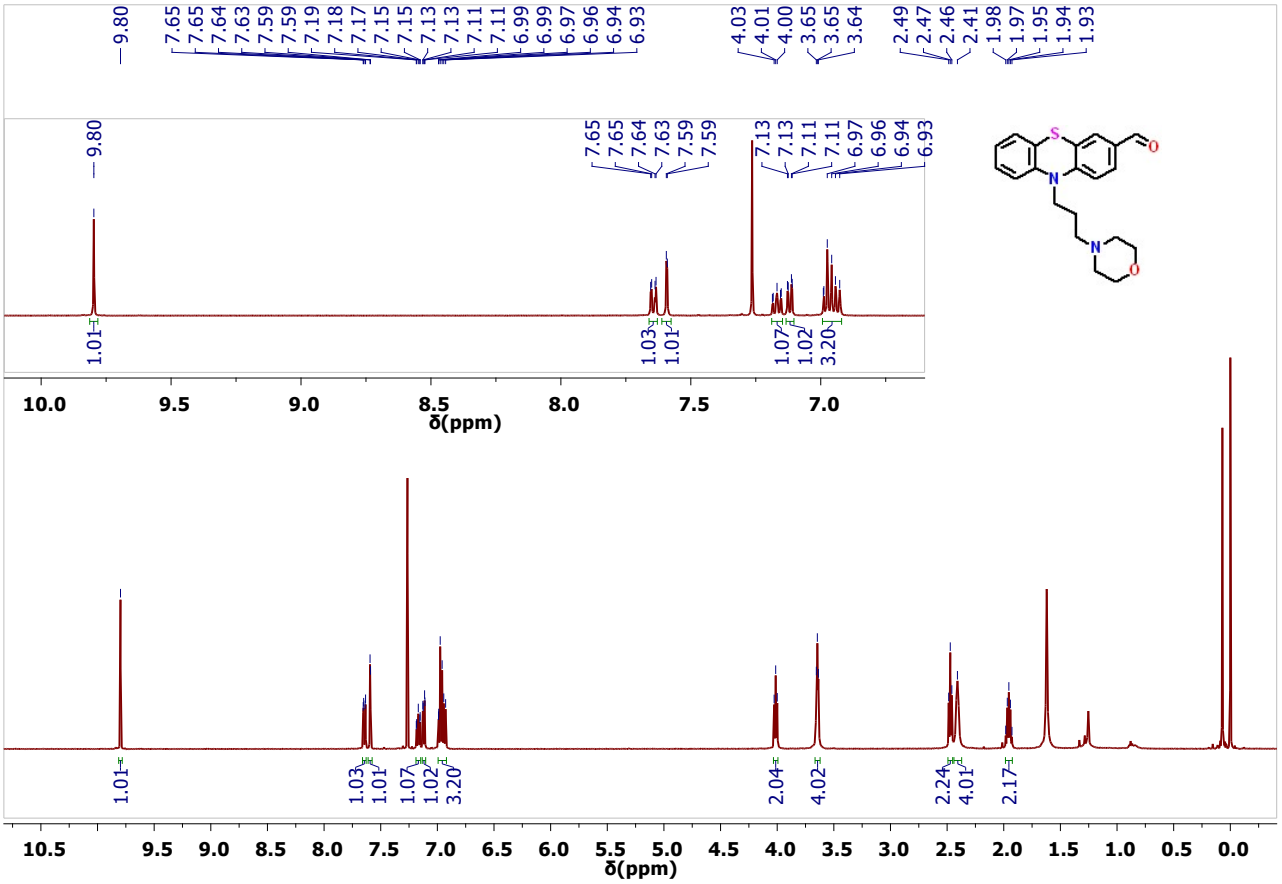


Figure S13: ^{1}H NMR spectra of **PTZ3** in $CDCl_3$.

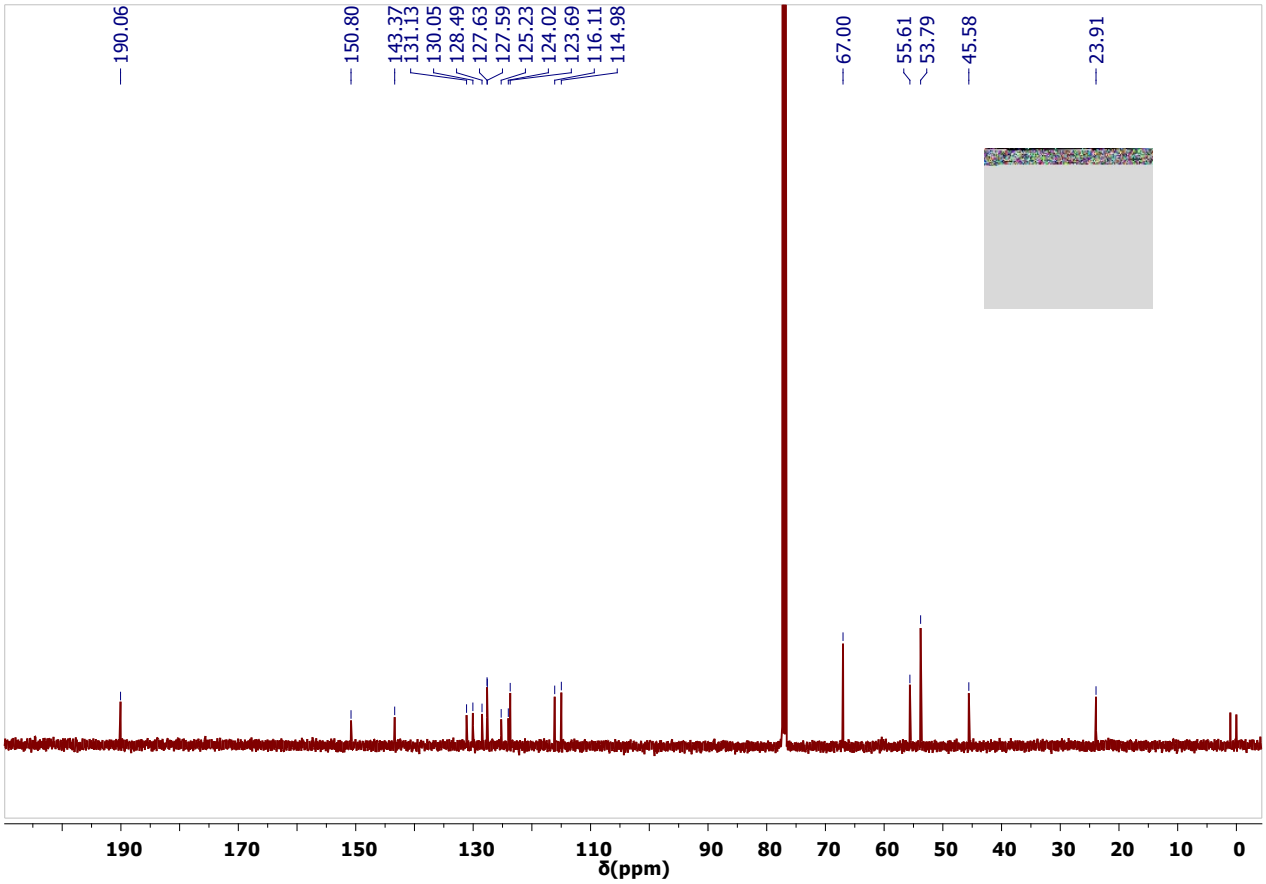


Figure S14: ^{13}C NMR spectra of **PTZ3** in $CDCl_3$.

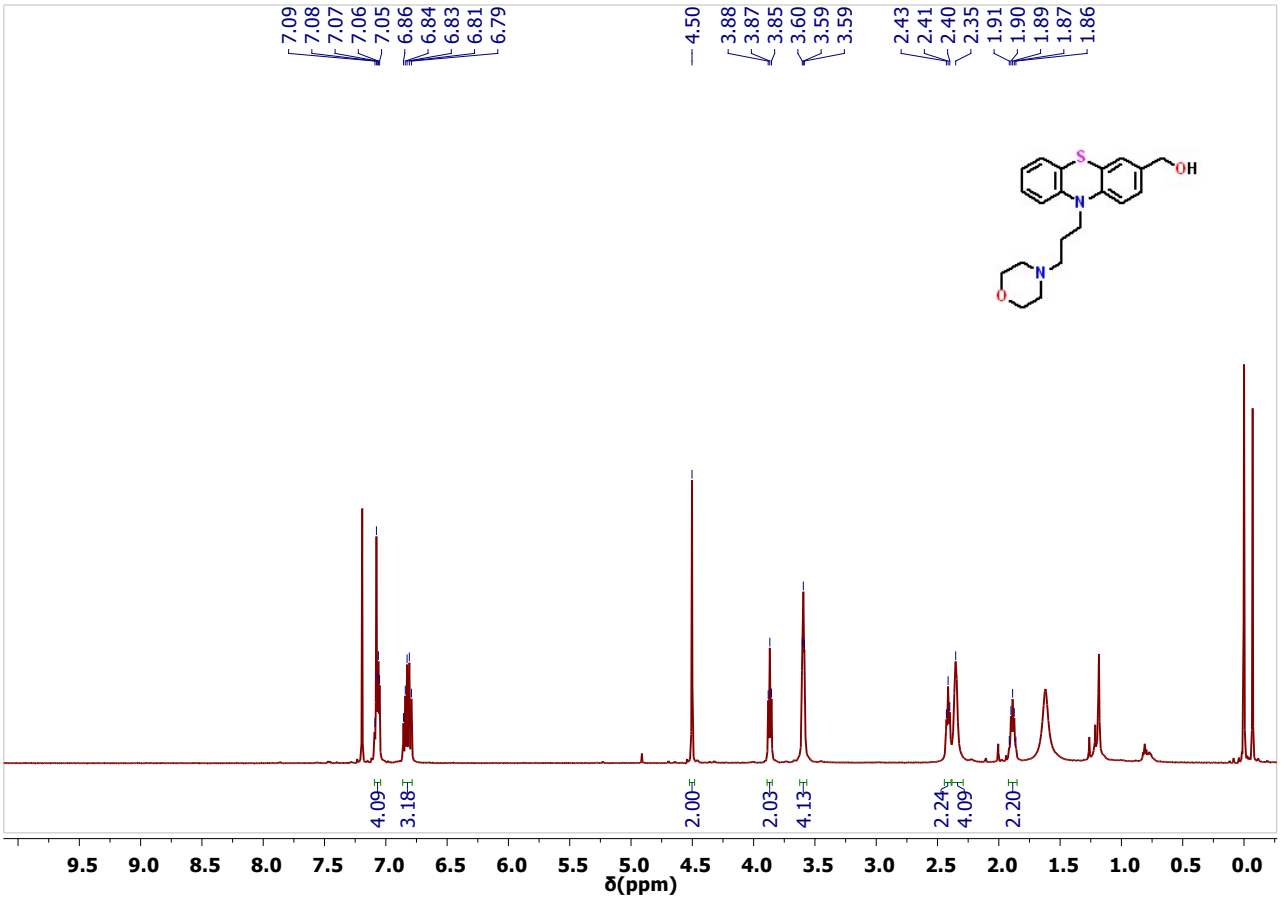


Figure S15: ^{1}H NMR spectra of **PTZ5** in $CDCl_3$.

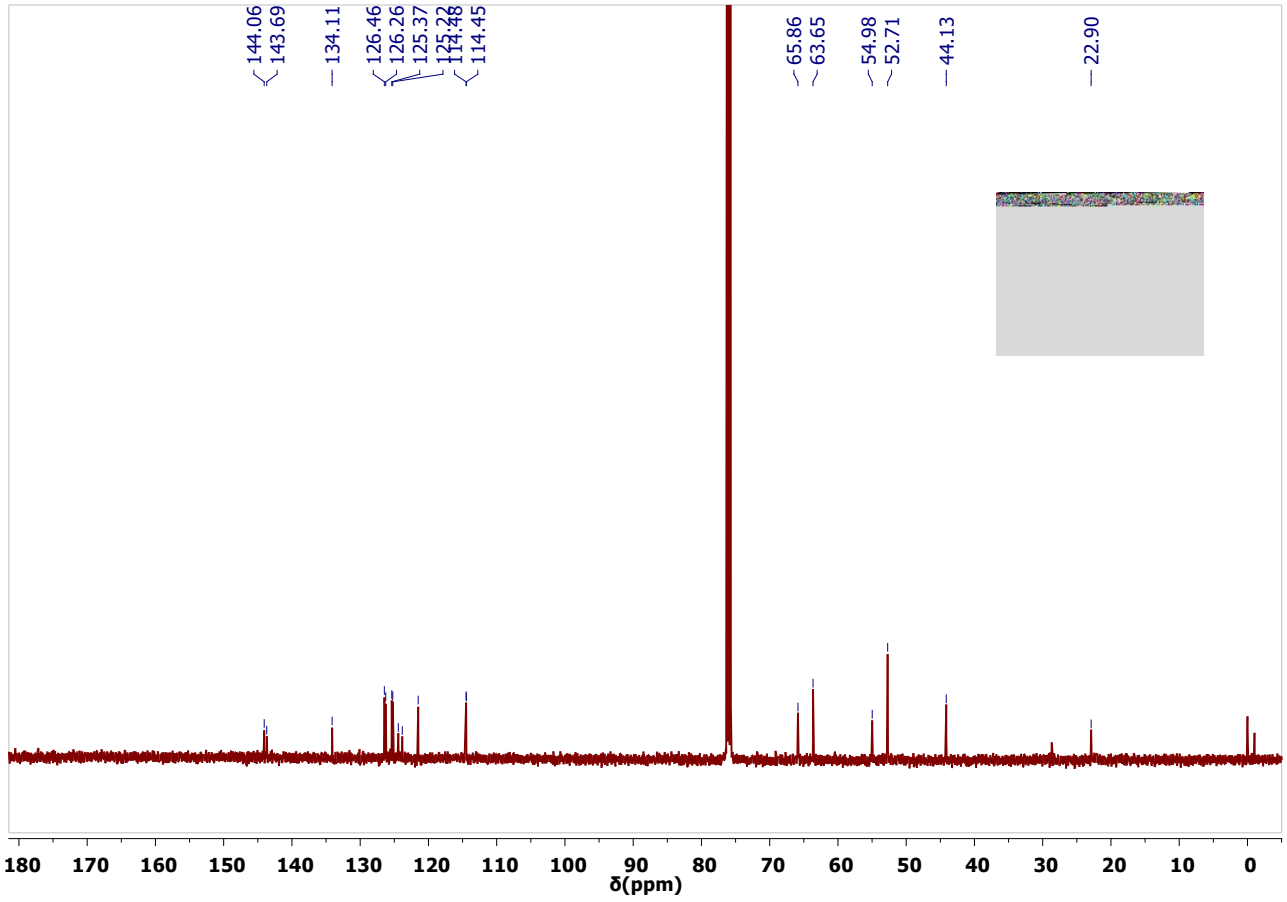


Figure S16: ^{13}C NMR spectra of **PTZ5** in $CDCl_3$.

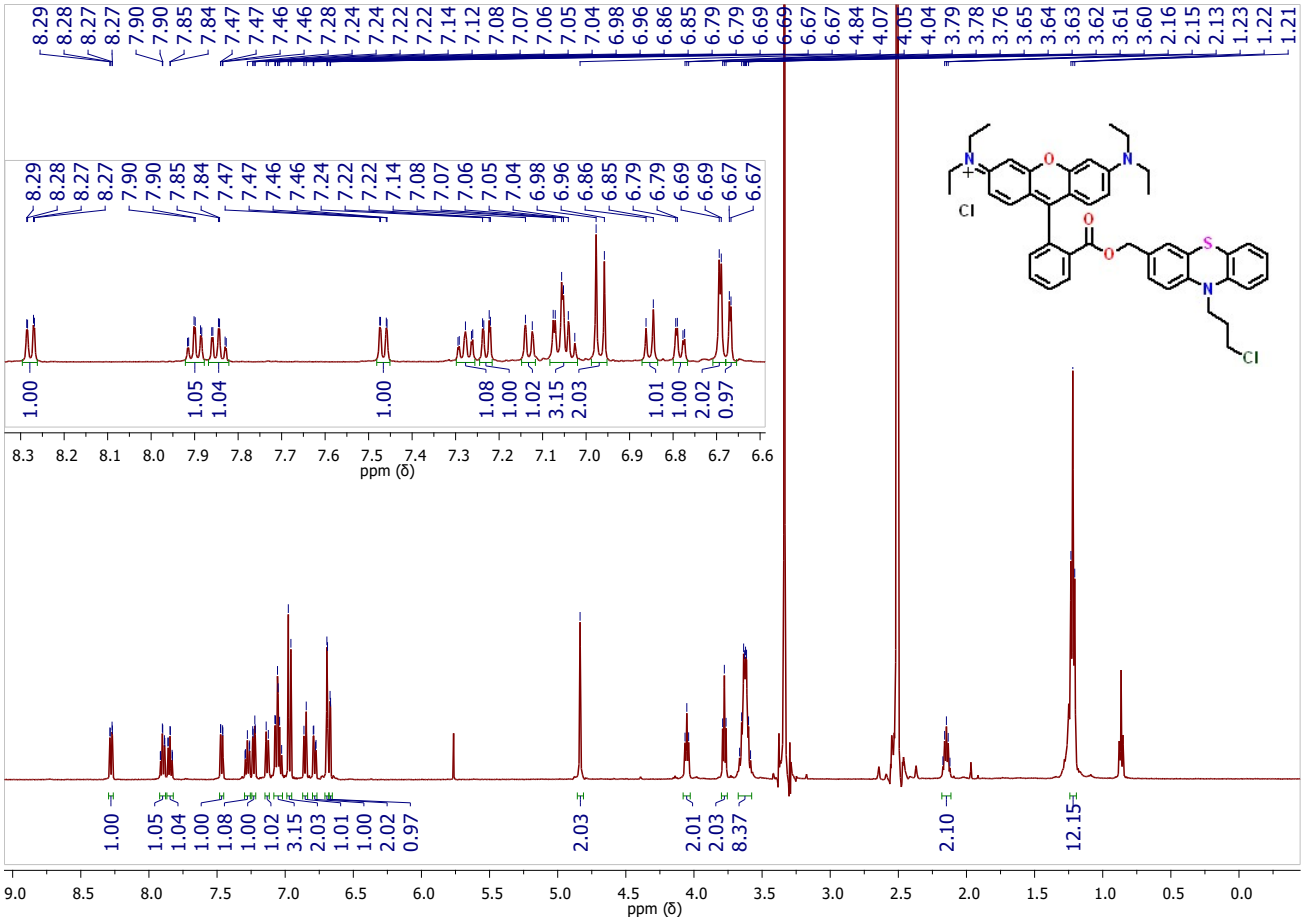


Figure S17: ^{1}H NMR spectra of **RP1** in DMSO-D_6.

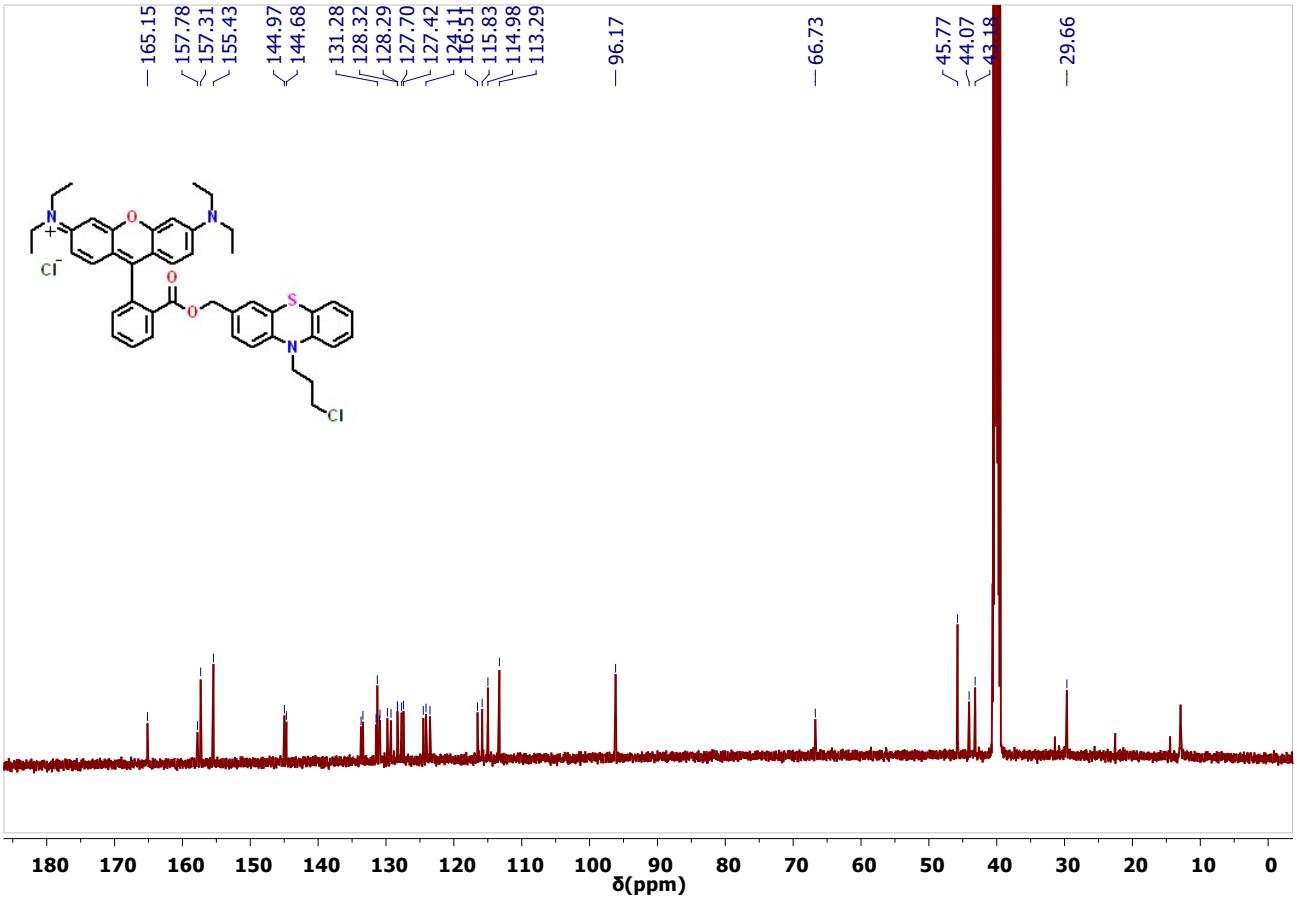


Figure S18: ^{13}C NMR spectra of **RP1** in DMSO-D_6.

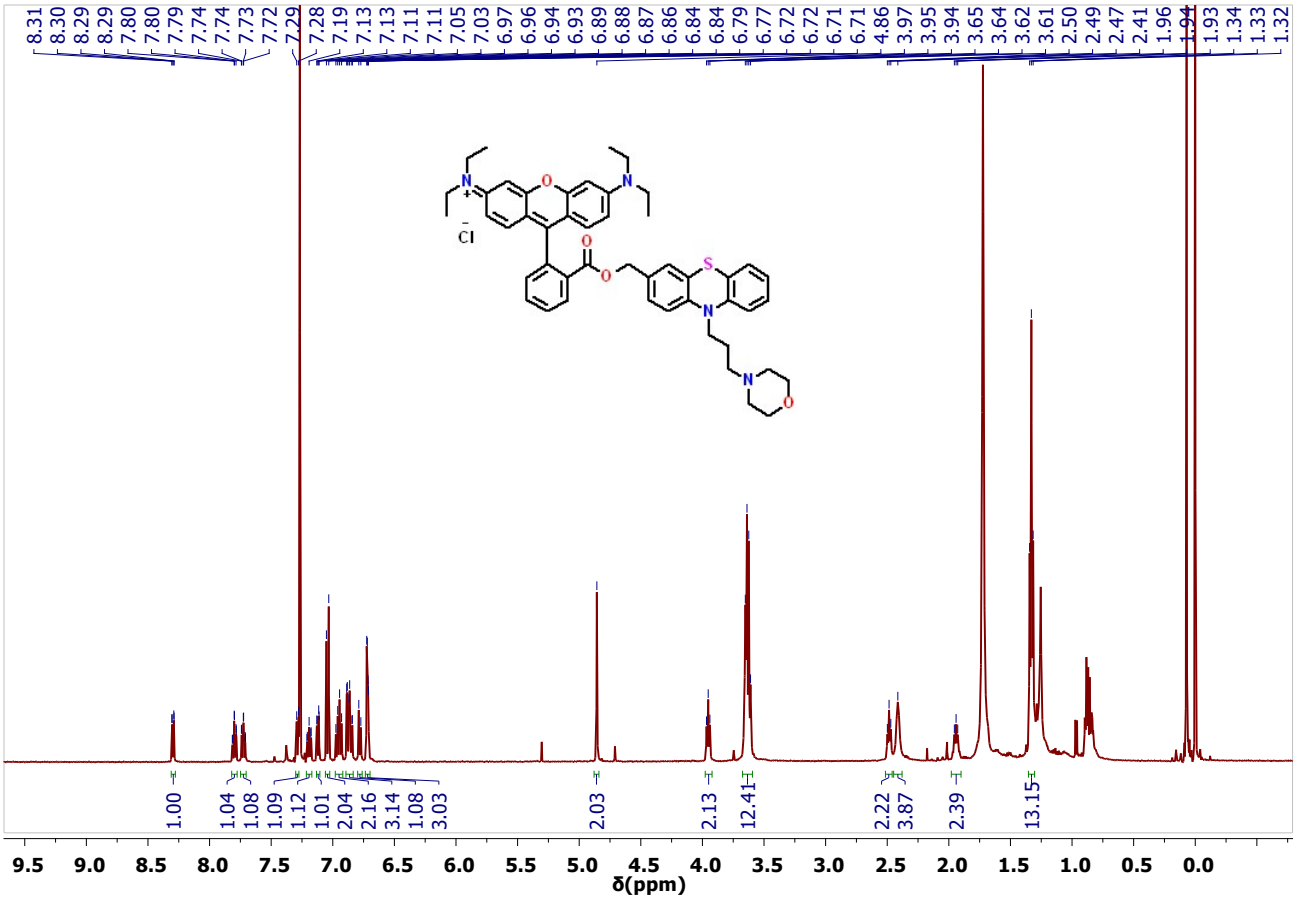


Figure S19: ^{1}H NMR spectra of **RP2** in $CDCl_3$.

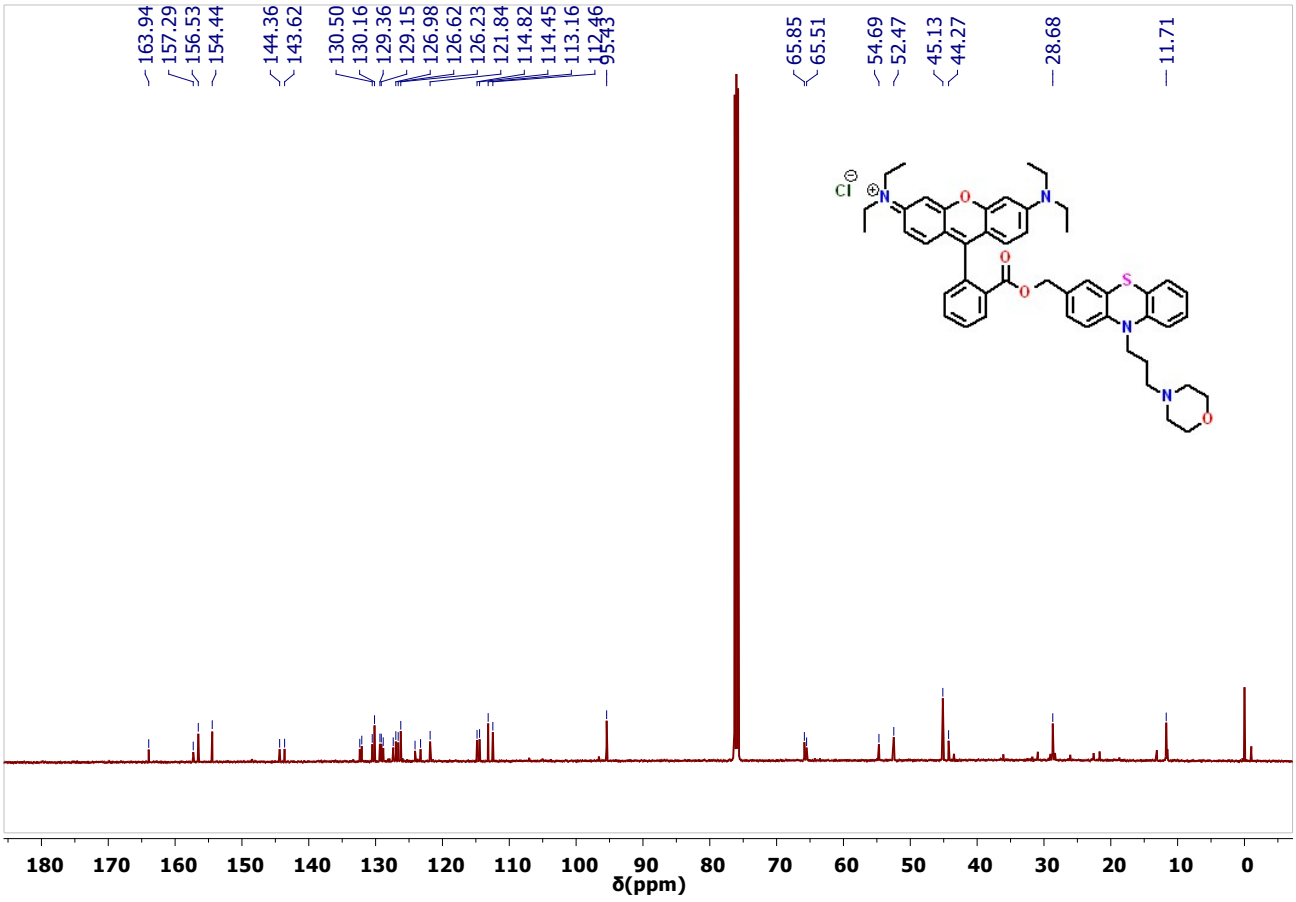


Figure S20: ^{13}C NMR spectra of **RP2** in $CDCl_3$.

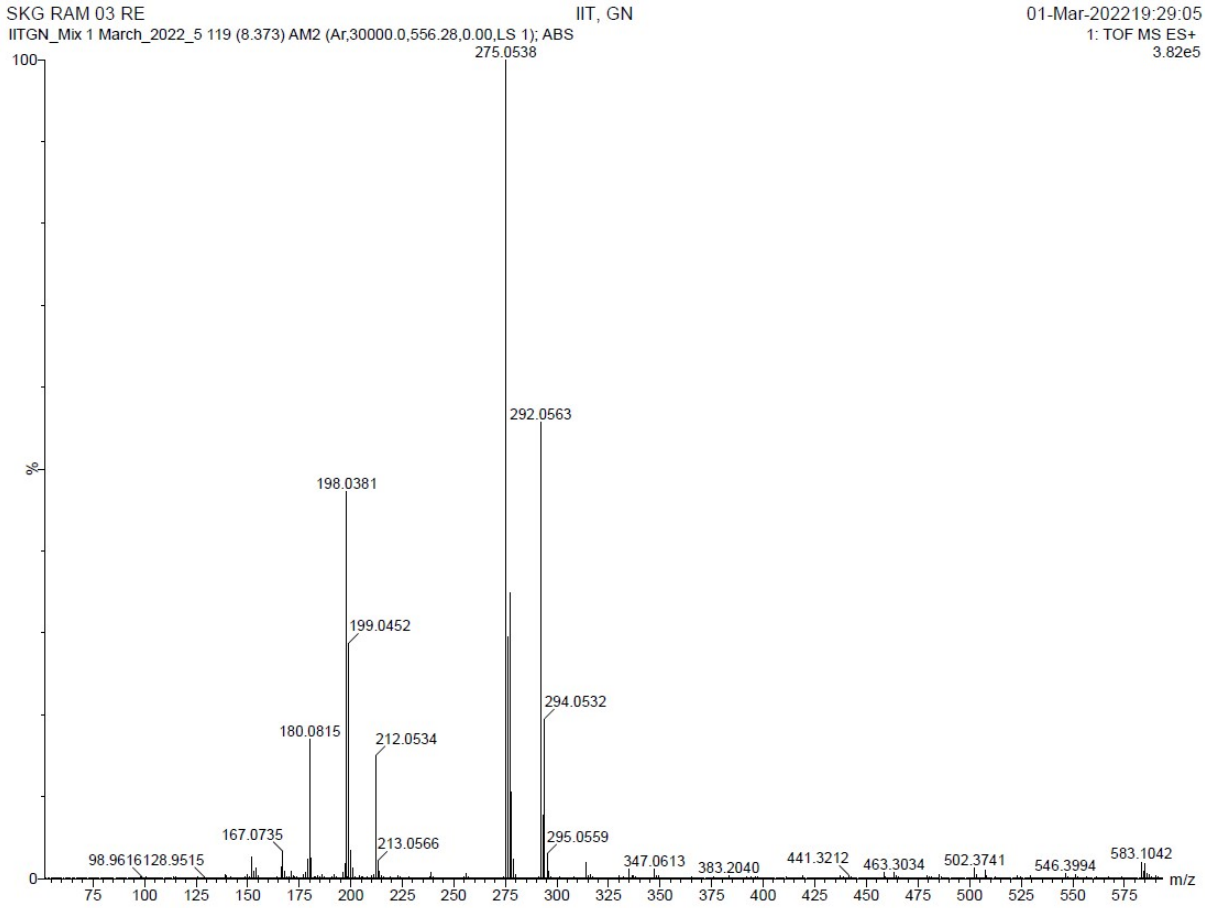


Figure S21: HR-MS (ESI-ToF) spectra of **PTZ1**.

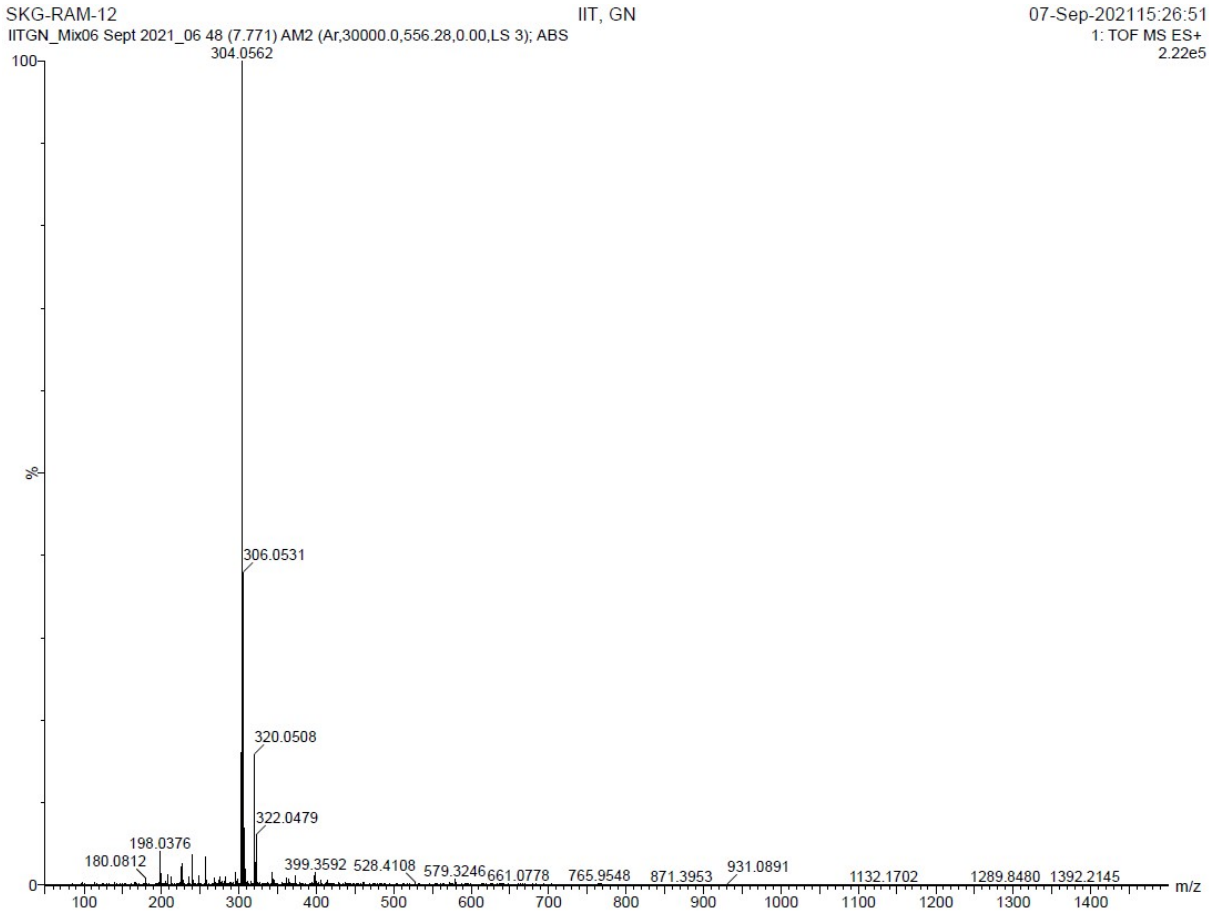


Figure S22: HR-MS (ESI-ToF) spectra of **PTZ2**.

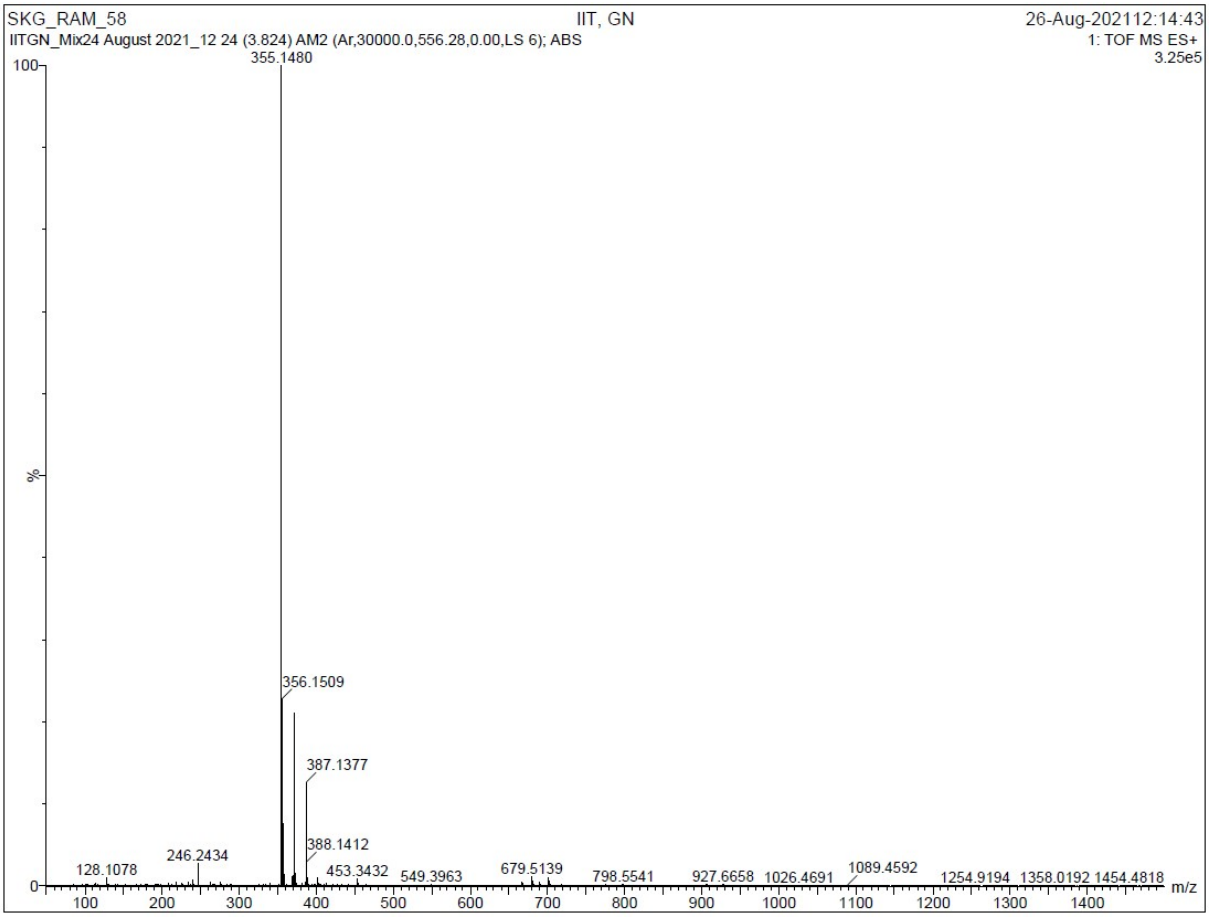


Figure S23: HR-MS (ESI-ToF) spectra of **PTZ3**.

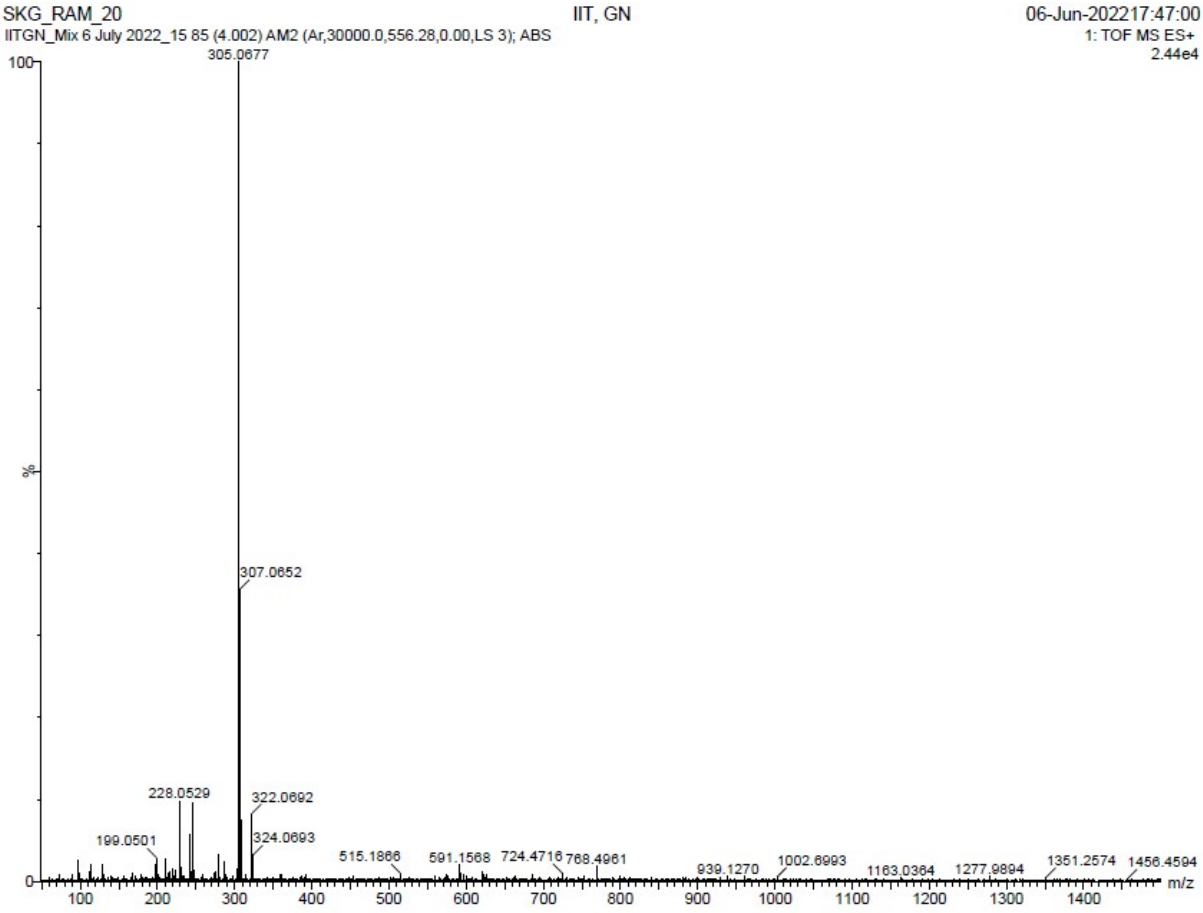


Figure S24: HR-MS (ESI-ToF) spectra of **PTZ4**.

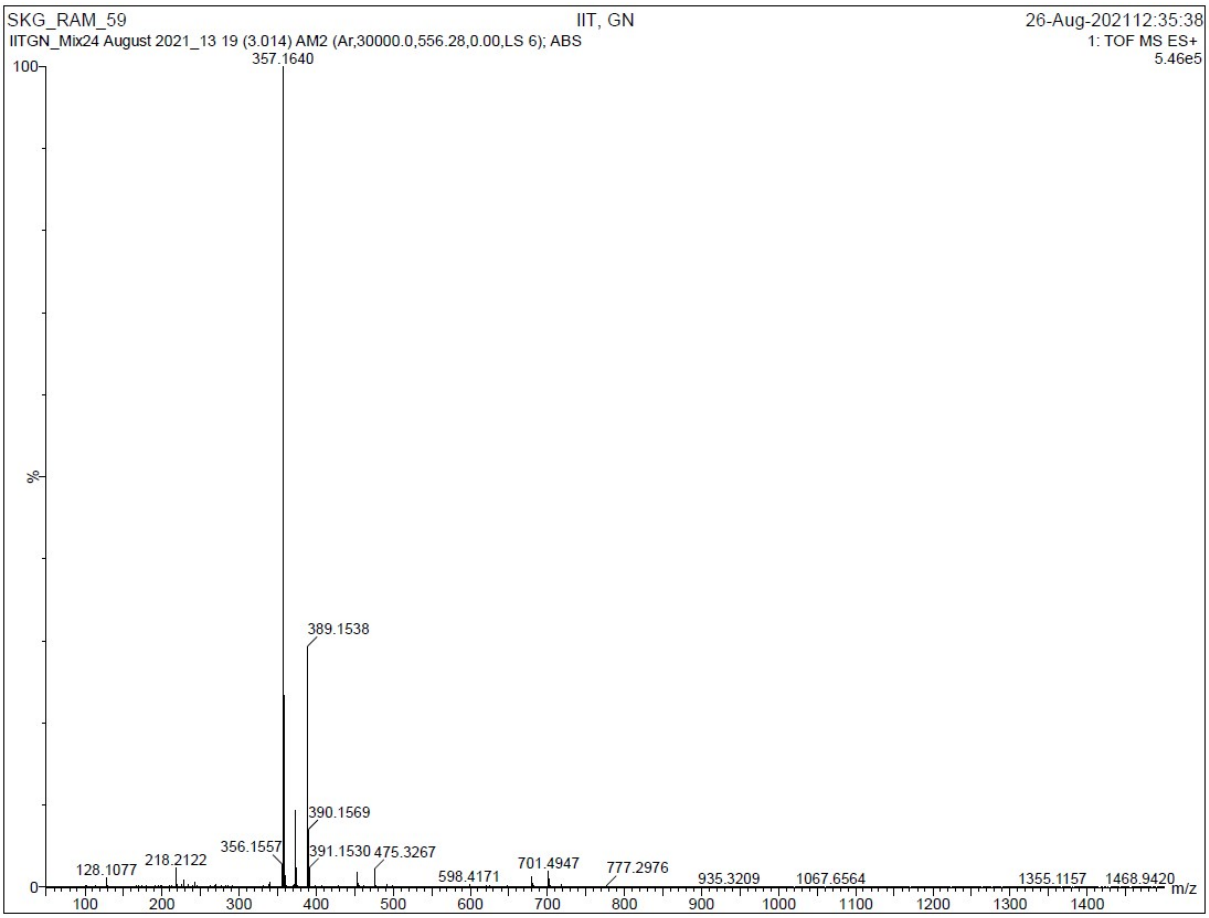


Figure S25: HR-MS (ESI-ToF) spectra of **PTZ5**.

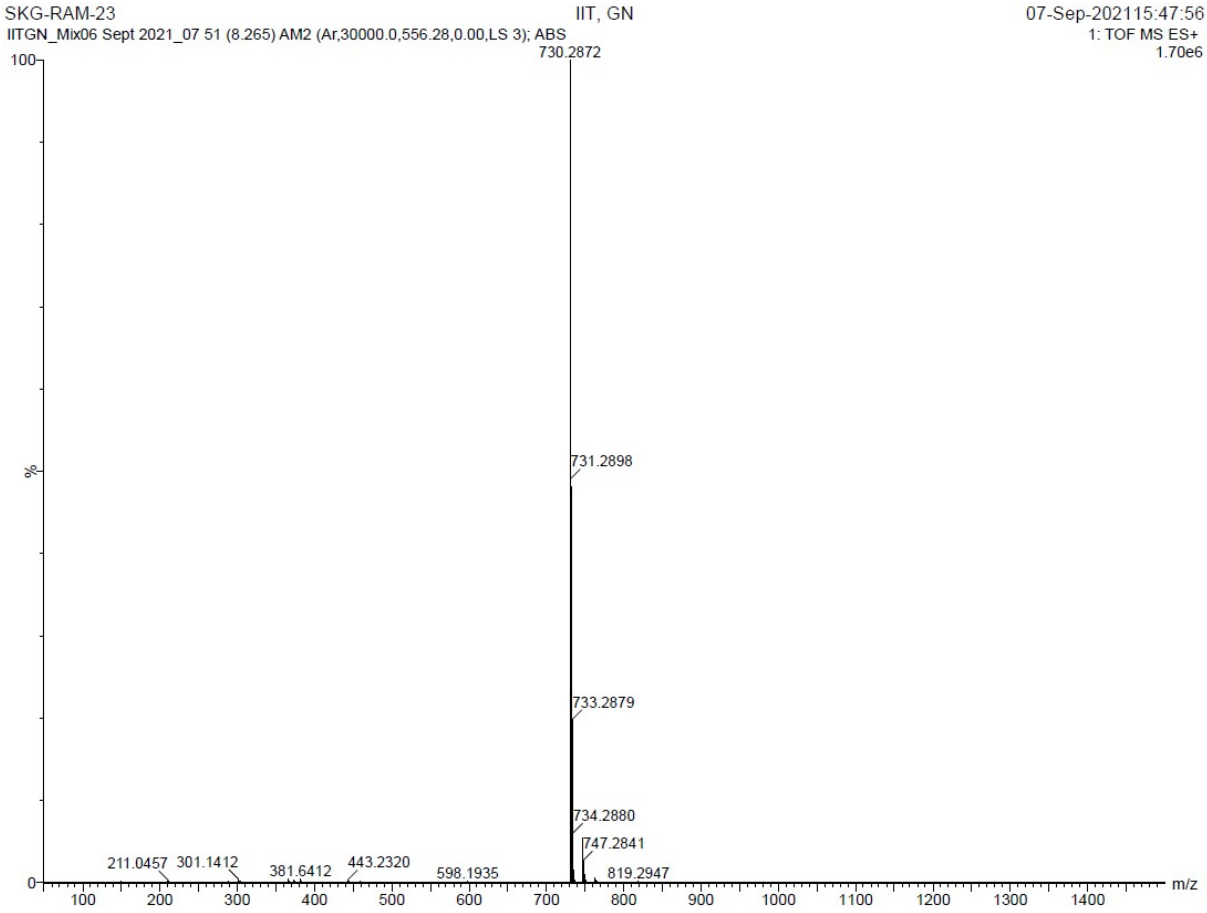


Figure S26: HR-MS (ESI-ToF) spectra of **RP1**.

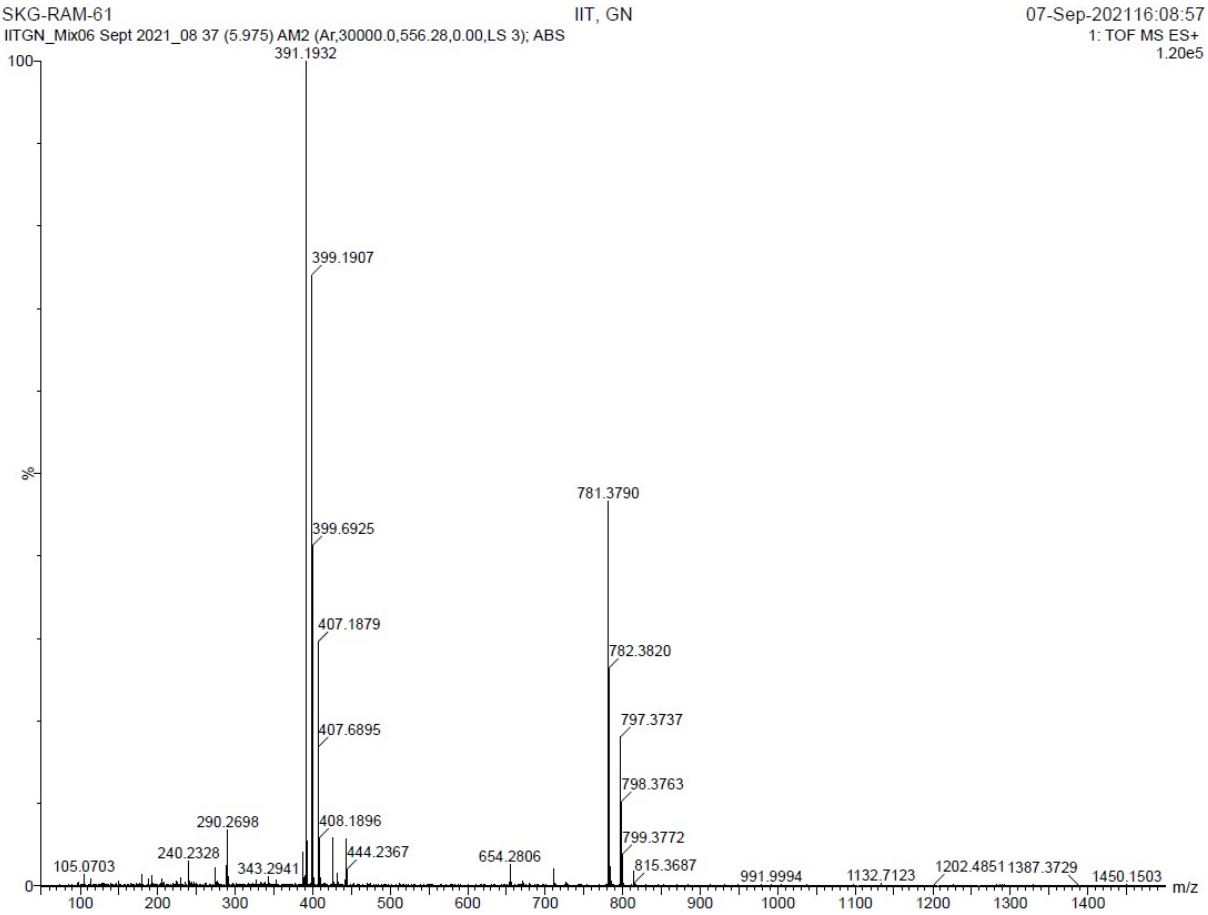


Figure S27: HR-MS (ESI-ToF) spectra of **RP2**.