Supporting information

Live Cell Imaging of Lipid Droplets: Fluorescent Chalcones as Probes for Lipophagy and Lipid-Mitochondria Interactions

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Synthesis and characterization details of the compounds

Synthetic procedure *3-Acetyl-7-(N, N-diethylamino)-2H-chromen-2-one*:

N,N-Diethylamino salicylaldehyde (1.93 g, 1 equiv) and methyl acetoacetate (1.16 g, 1.2 equiv) were dissolved in ethanol (10 mL) in a 50 mL round-bottom flask. Piperidine (2 equiv) was then added, and the reaction mixture was refluxed for 4 h while monitoring the progress by thin-layer chromatography (TLC). Upon completion, the reaction mixture was concentrated using a rotary evaporator and cooled to yield a yellow solid. The crude product was recrystallized from methanol to afford 3-acetyl-7-(N,N-diethylamino)-2H-chromen-2-one as a yellow solid (1.81 g, 90 % yield) **^{1}H NMR (500 MHz, DMSO)** δ 8.50 (s, 1H), 7.67 (d, *J* = 9.0 Hz, 1H), 6.80 (d, *J* = 9.0 Hz, 1H), 6.57 (s, 1H), 3.49 (q, *J* = 7.0 Hz, 4H), 3.34 (s, 3H), 1.14 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR (126 MHz, DMSO)** δ 194.69, 160.36, 158.75, 153.43, 148.14, 132.91, 115.46, 110.60, 107.95, 96.27, 44.88, 30.62, 12.8.

M1: ***(2E,4E)-5-(4-(dimethylamino)phenyl)-1-(6-methoxynaphthalen-2-yl)penta-2,4-dien-1-one:***

1-(6-Methoxynaphthalen-2-yl)ethan-1-one (150 mg, 1 equiv) was dissolved in a 10 mL methanol:dioxane mixture (2:1) in a dry round-bottom flask. The solution was refluxed for 30 minutes, after which 2% aqueous NaOH (1 mL) was added. The mixture was stirred for an additional 30 minutes. Subsequently, (E)-3-(4-(dimethylamino)phenyl)acrylaldehyde (131.27 mg, 1 equiv) was added, and the reaction mixture was refluxed for 12 h. After cooling the reaction mixture in an ice bath, the resulting precipitate was collected by filtration and purified by recrystallization from methanol Orange-red colour solid Yield: 227.6 mg 85%, **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.43 (s, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.69 (dd, *J* = 14.7 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.20 (d, *J* = 8.9 Hz, 1H),

7.16 (dd, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 15.3 Hz, 1H), 6.90 (dd, *J* = 15.3 Hz, 1H), 6.68 (t, *J* = 5.8 Hz, 2H), 3.95 (s, 3H), 3.02 (s, 6H). **^{13}C NMR (125 MHz, $CDCl_3$)** δ 189.94, 159.52, 151.09, 145.93, 142.87, 136.97, 134.10, 131.09, 129.48, 128.89, 127.98, 127.10, 125.30, 124.33, 122.67,122.61, 119.56, 112.07, 105.77, 55.44, 40.24. **HRMS:** Actual mass**:** 358.1807, Observed mass: 358.1818, Mass error: 3.07 ppm.

M2: ***(2E,4E,6E)-7-(4-(dimethylamino)phenyl)-1-(6-methoxynaphthalen-2-yl)hepta-2,4,6-trien-1-one:***

1-(6-Methoxynaphthalen-2-yl)ethan-1-one (200 mg, 1 equiv) was dissolved in 10 mL of a methanol:dioxane mixture (2:1) in a dry round-bottom flask. The solution was refluxed for 30 minutes, followed by the addition of 2% aqueous NaOH. The reaction mixture was heated for an additional 30 minutes. Subsequently, (2E,4E)-5-(4-(dimethylamino)phenyl)penta-2,4-dienal (200 mg, 1 equiv) was added, and the mixture was refluxed for 12 h. After cooling, the precipitate was collected by filtration and purified by recrystallization from methanol . Dark red solid, Yield 114 mg, 40%; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.42 (d, *J* = 7.3 Hz, 1H), 8.04 (d, *J*=8.5 Hz, 1H), 7.86 7.85 (d, *J*=8.9 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.61 (dd, *J* = 14.7 Hz, 1H), 7.36 (d, *J* = 8.7 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 1H), 7.16 (s, 1H), 7.12 (d, *J* = 14.7 Hz, 1H), 6.86 (dd, *J* = 14.4 Hz, 1H), 6.86 (dd, *J* = 14.3 Hz, 1H), 6.68 (t, *J* = 6.2 Hz, 2H), 6.54 (dd, *J* = 14.4 Hz, 1H), 3.95 (s, 3H), 2.99 (s, 6H). **^{13}C NMR (125 MHz, $CDCl_3$)** δ 188.83, 158.54, 149.65, 144.08, 142.68, 137.03, 135.98, 132.97, 130.07, 128.48, 127.65, 127.32, 126.94, 126.09, 124.23, 123.85, 122.94, 122.40, 118.55, 111.17, 104.76, 54.40, 39.26. **HRMS:** Actual mass**:** 384.1963, Observed mass: 358.1979, Mass error: 4.16 ppm

M3: ***7-(diethylamino)-3-((2E,4E)-5-(4-(dimethylamino)phenyl)penta-2,4-dienoyl)-2H-chromen-2-one,***

3-Acetyl-7-(diethylamino)-2H-chromen-2-one (150 mg, 1 equiv) was dissolved in ethanol (10 mL) in a dry round-bottom flask. The solution was refluxed for 30 minutes, followed by the addition of piperidine (200 µL, 2 equiv). Refluxing was continued for another 30 minutes, after which (E)-3-(4-(dimethylamino)phenyl)acrylaldehyde (123 mg, 1 equiv) was added to the reaction mixture. The contents were refluxed for 8 h, then cooled to room temperature. The resulting precipitate was collected by filtration and purified by recrystallization from ethanol . Dark red solid, Yield 132 mg, 87%, **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.52 (s, 1H), 7.67 (d, *J*= 14.55, 1H), 7.65 (d, *J*=14.25, 1H), 7.58 (d, *J* = 14.8 Hz, 1H), 7.40 (d, *J* = 16.7 Hz, 2H), 7.39 (s, 1H), 6.92 (d, *J* = 8.2 Hz, 2H), 6.67 (d, *J* = 8.7 Hz, 2H), 6.61 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.48

(d, *J* = 1.9 Hz, 1H), 3.45 (q, *J* = 7.1 Hz, 4H), 3.01 (s, 6H), 1.24 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR (125 MHz, $CDCl_3$)** δ 186.27, 160.93, 158.50, 152.72, 151.00, 148.29, 145.29, 142.59, 131.62, 128.88, 125.69, 124.62, 123.49, 117.33, 112.08, 109.71, 108.72, 96.64, 45.15, 40.25, 12.49. **HRMS:** Actual mass**:** 417.2178, Observed mass: 417.2171, Mass error: -1.68 ppm

Absorption spectra of **M1** and **M3**

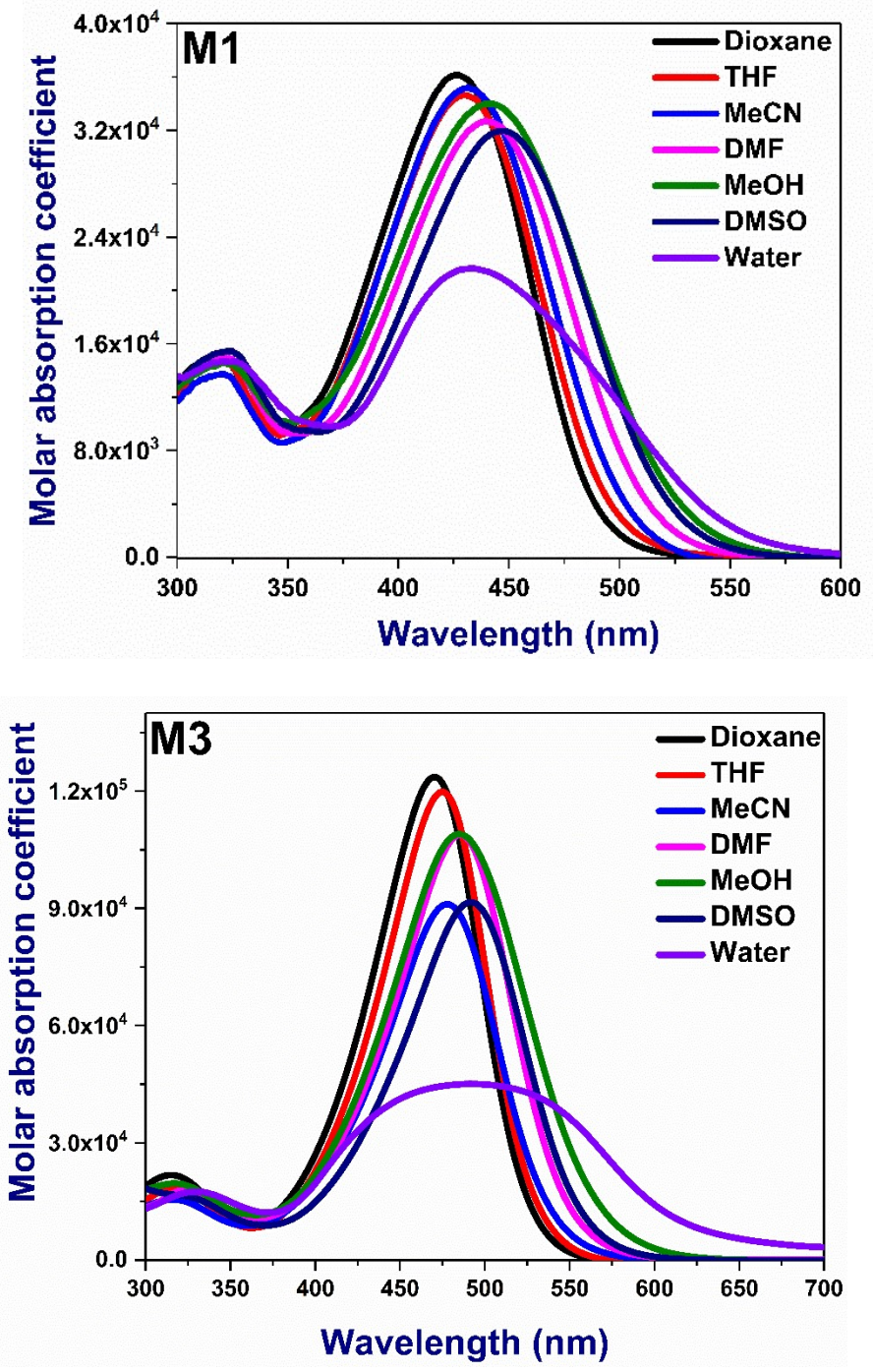


Figure S1: Absorption spectrum of **M1** and **M3** in various solvents

Emission spectra of M1 and M3

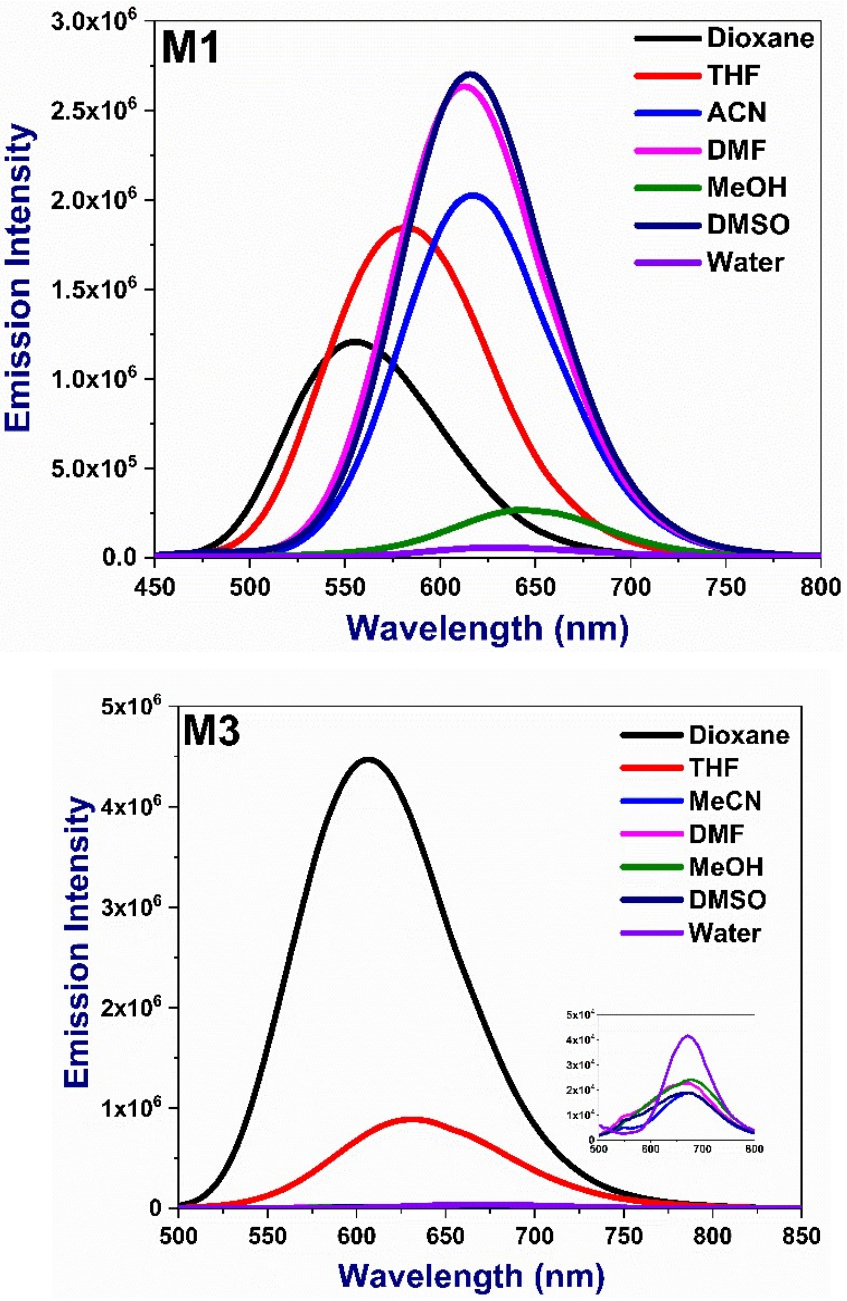


Figure S2: Emission spectra of **M1** and **M3** in various solvents; inset of **M3** shows the zoomed spectra in polar solvents (MeCN, DMF, MeOH, DMSO, Water).

Binary mixture investigation

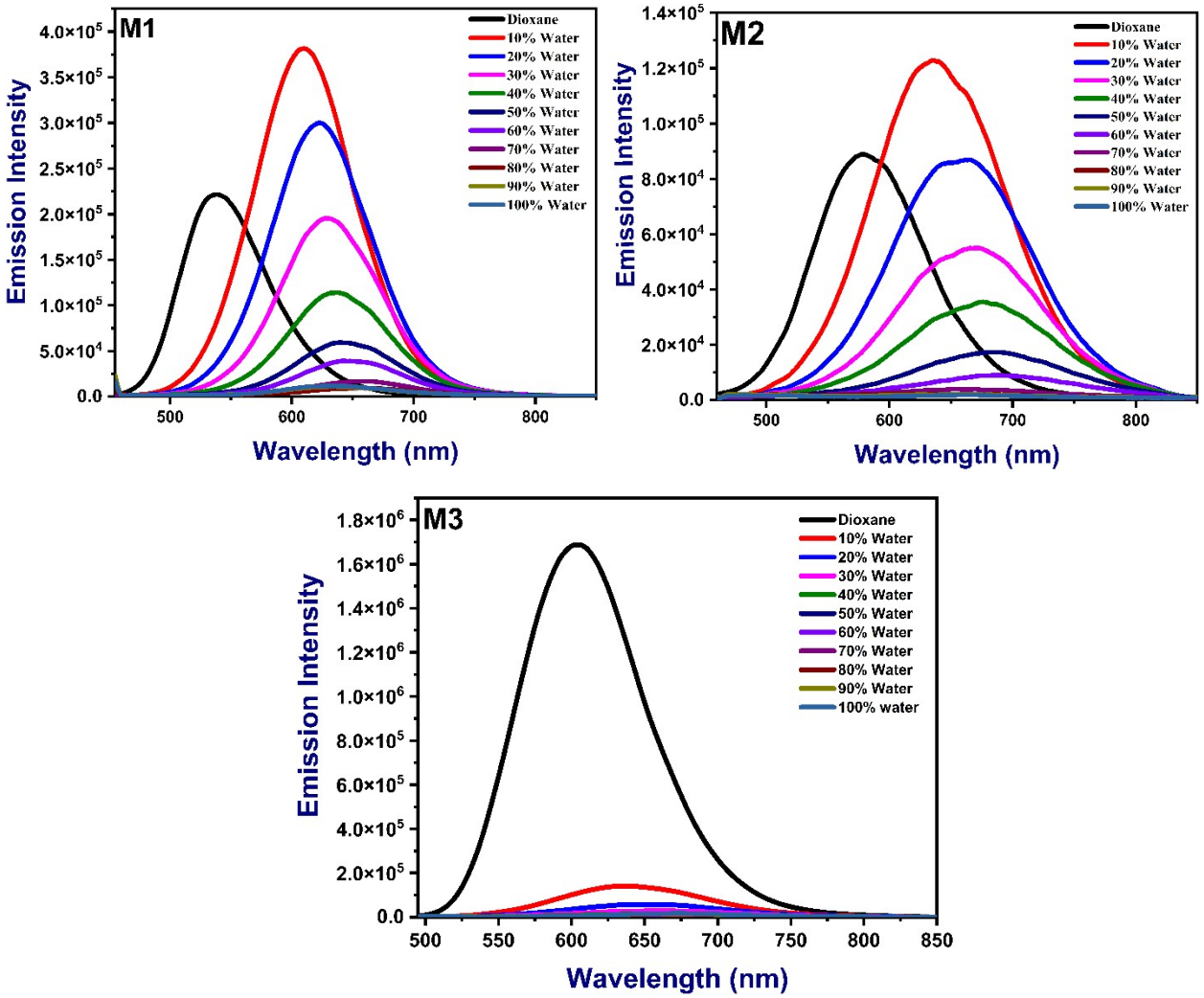


Figure S3: Emission Spectra of **M1**, **M2** and **M3** in dioxane: water binary solvent mixture.

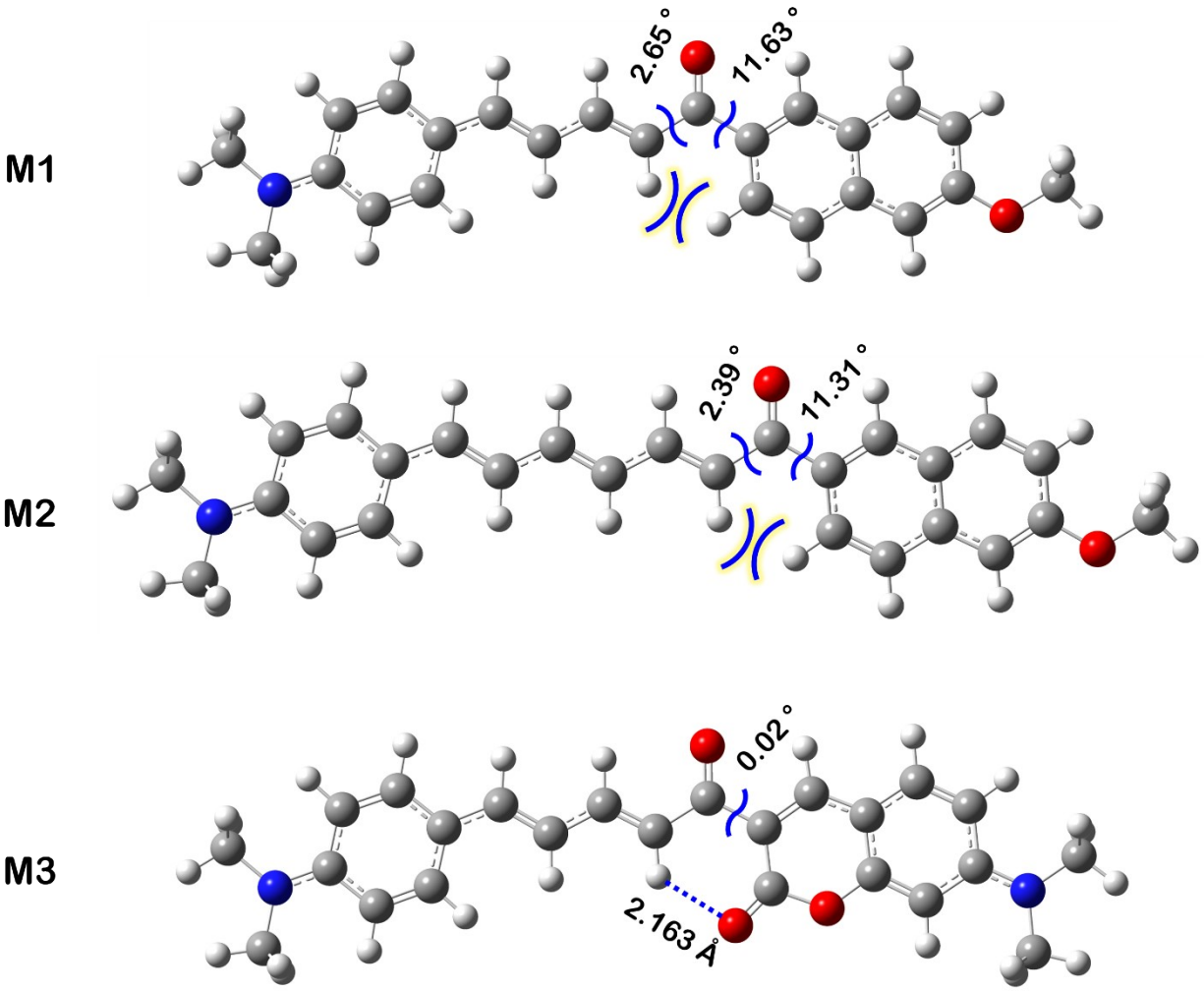


Fig. S4. Optimized molecular geometries of the chalcone derivatives (**M1**-**M3**) with preferred torsional distortions and intramolecular contacts.

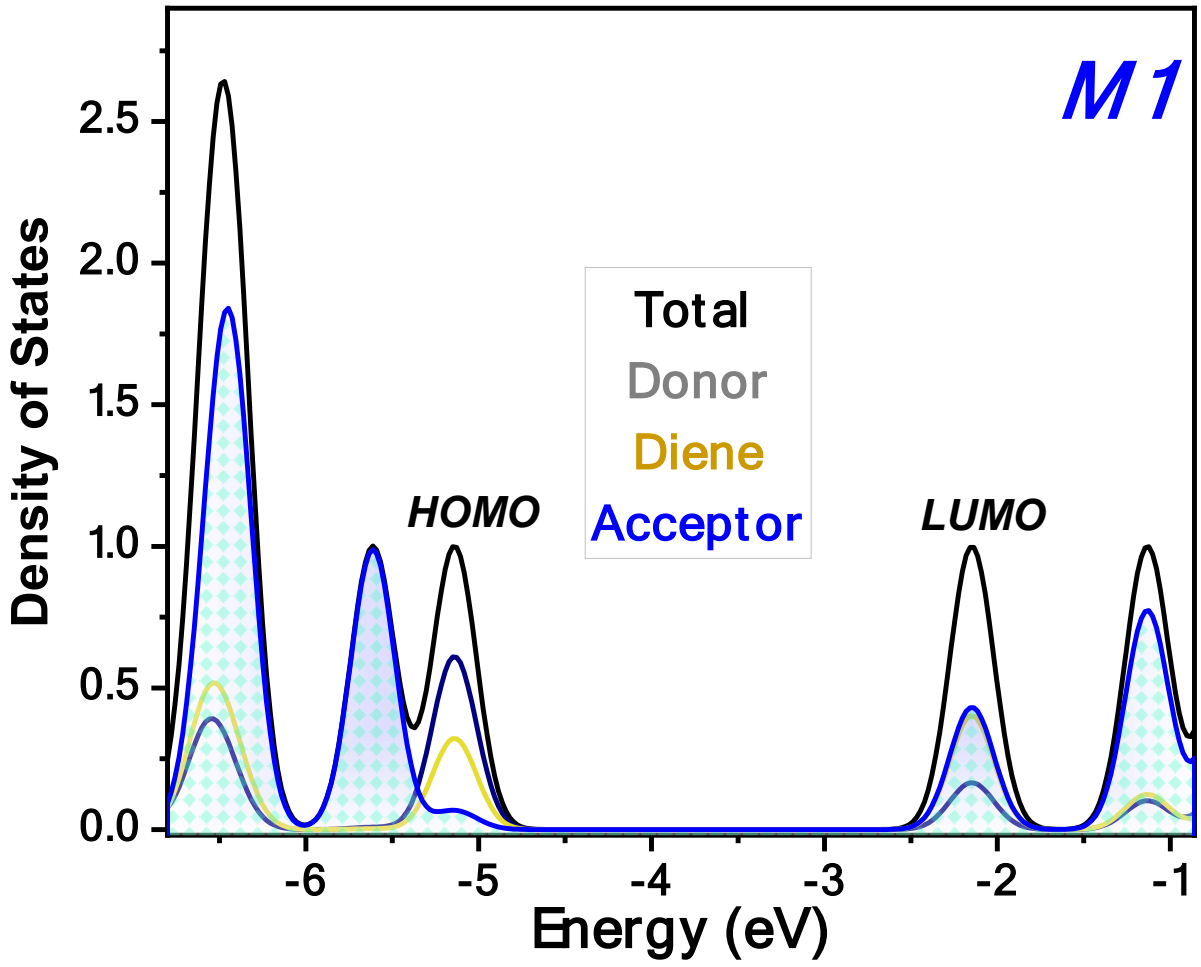


Fig. S5 DOS-PDOS analysis of **M1** shows the impact of the π-spacer and acceptor units on the HOMO and LUMO electron distribution in terms of shape broadening and energetic shift obtained from DFT/B3LYP/6-311G (d, p) level of theory

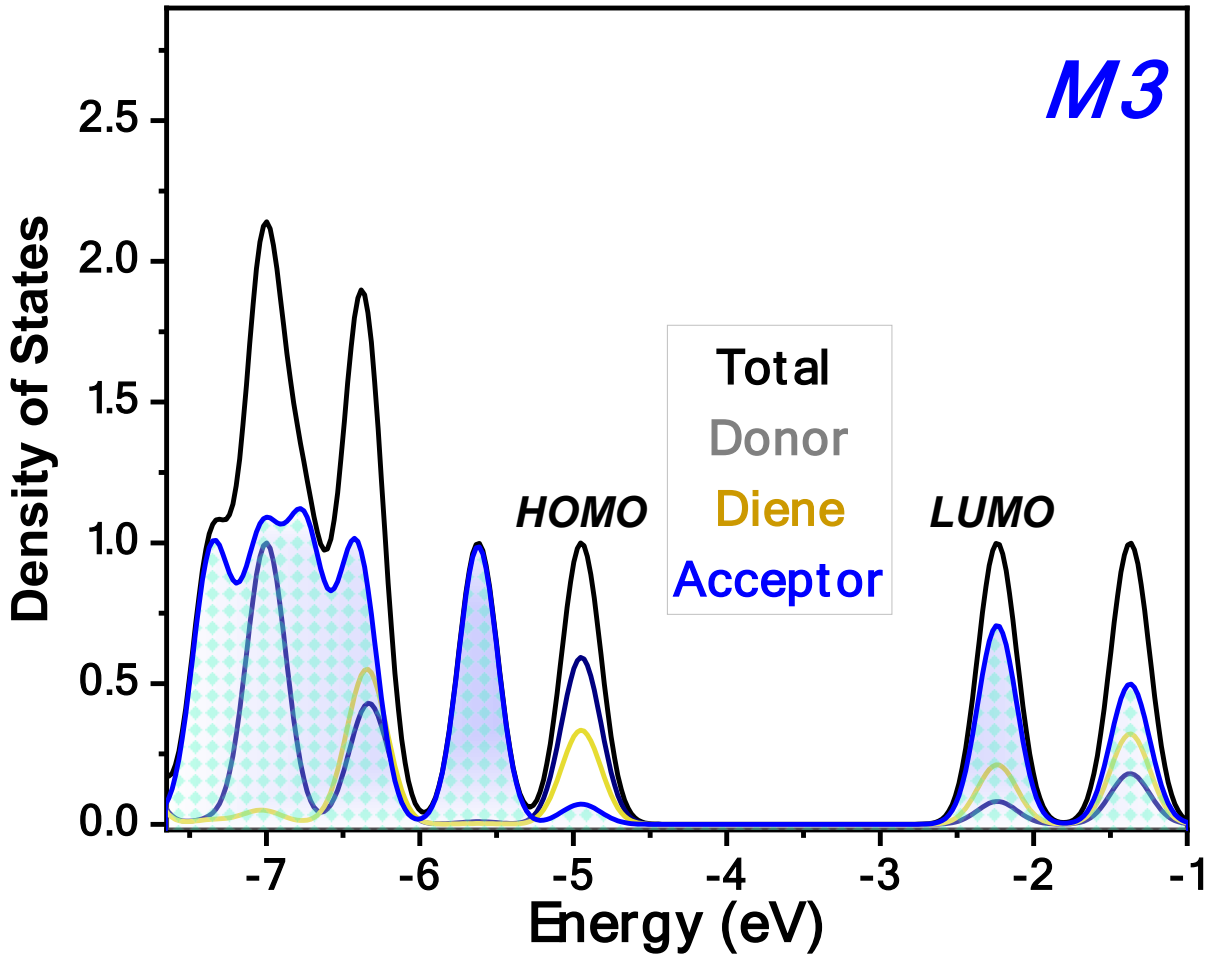


Fig S6. DOS-PDOS analysis of **M3** shows the impact of the π-spacer and acceptor units on the HOMO and LUMO electron distribution in terms of shape broadening and energetic shift obtained from DFT/B3LYP/6-311G (d, p) level of theory.

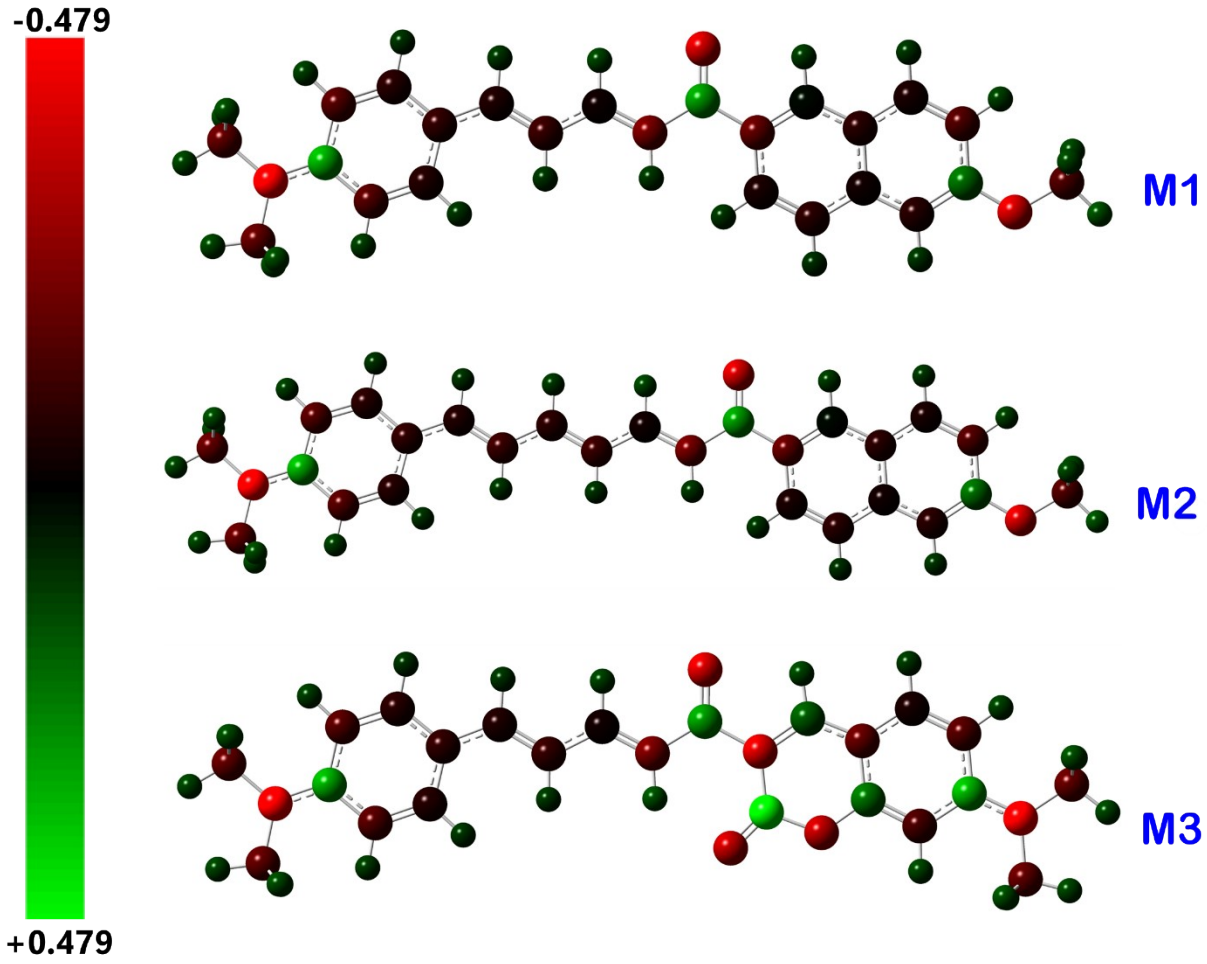
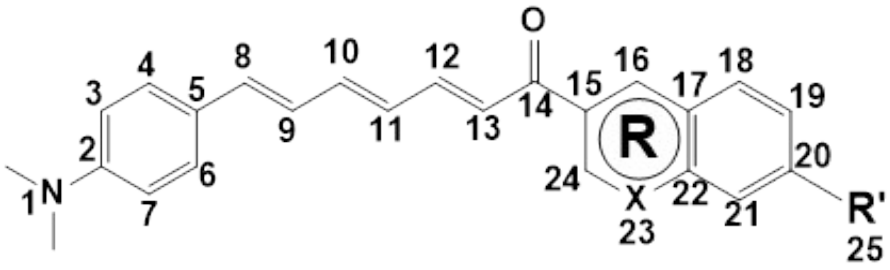


Fig S7. Mulliken population analysis of the chalcone derivatives reveals the charge density variation on the acceptor segments.



	M1	M2	M3
N_1-C_2	1.367	1.368	1.367
C_2-C_3	1.419	1.422	1.419
C_3-C_4	1.382	1.380	1.362
C_4-C_5	1.410	1.412	1.410
C_5-C_6	1.412	1.410	1.412
C_6-C_7	1.380	1.382	1.380
C_2-C_7	1.422	1.418	1.422
C_5-C_8	1.445	1.446	1.446
C_8-C_9	1.361	1.362	1.361
C_9-C_{10}	1.429	1.428	1.430
C_{10}-C_{11}	1.359	1.365	1.359
C_{11}-C_{12}	-	1.427	-
C_{12}-C_{13}	-	1.359	-
C_{13}-C_{14}	1.466	1.467	1.464
C_{14}-C_{15}	1.501	1.501	1.501
C_{15}-C_{16}	1.383	1.383	1.377
C_{16}-C_{17}	1.412	1.412	1.408
C_{17}-C_{18}	1.418	1.418	1.415
C_{18}-C_{19}	1.374	1.374	1.372
C_{19}-C_{20}	1.421	1.421	1.432
C_{20}-C_{21}	1.384	1.384	1.417
C_{21}-C_{22}	1.410	1.410	1.381
C_{22}-X_{23}	1.423	1.423	1.363
X_{23}-C_{24}	1.371	1.371	1.402
C_{24}-C_{15}	1.426	1.426	1.457
C_{20}-R_{25}	1.360	1.359	1.360

Fig S8: Geometrical coordinates of the molecules showing the bond length variation at the neutral states.

Cell toxicity study (MTT assay)

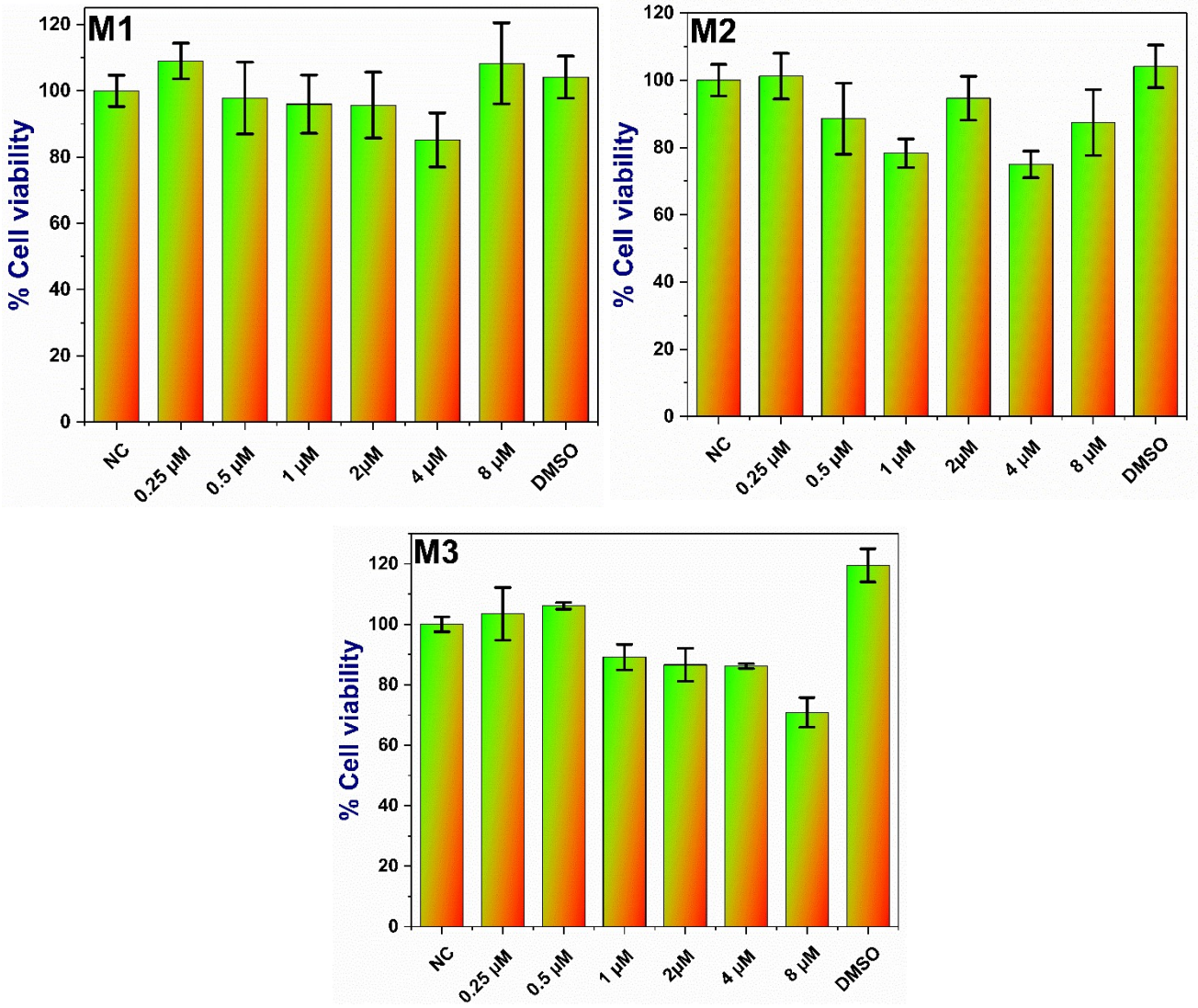


Figure S9: MTT assay results showing cell viability under different **M1-M3** concentrations over 48 h. Results are represented as a mean ± SD. Statistical analysis was performed using Origin 2017 64Bit software

Cellular imaging and colocalization study of **M1** in different cell lines.

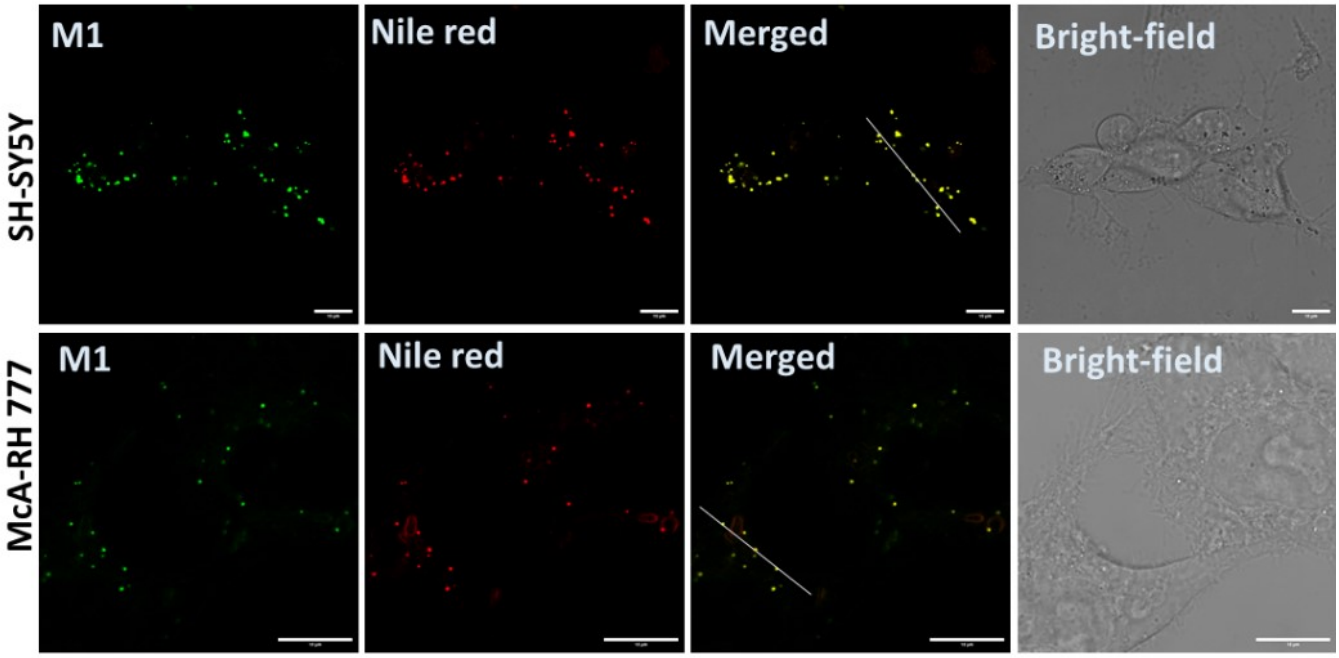


Figure S10A: Cellular imaging and colocalization of **M1** in SH-SY5Y and McARH 7777 cells, (**M1**= λ_{ex}- 458 nm, λ_{em}- 435-530nm, and Nile Red λ_{ex}- 514 nm, λ_{em} 525-565 nm; (incubation 10 min at 37 °C, 5% CO_2, Scale bar=10 μm). Green channel for **M1**, red channel for Nile Red, merged image for respective cells.

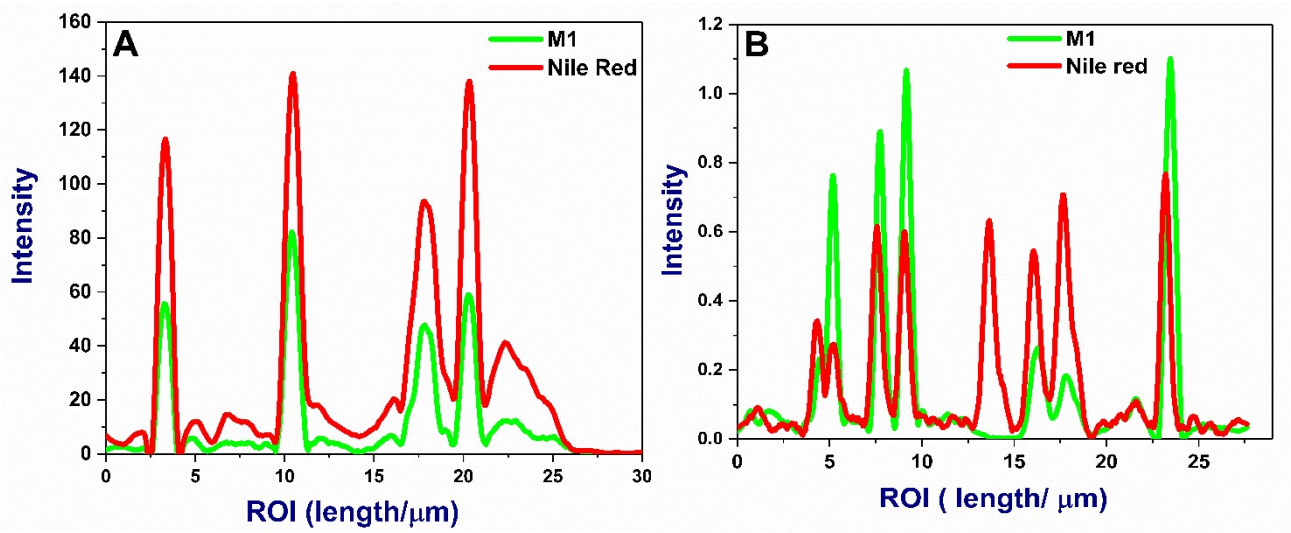


Figure S10B: Line profile graph of **M1** in SH-SY5Y **(A)** and McARH 7777 **(B)** cell lines.

Cellular imaging and colocalization of **M1** and **Nile Red** with **LysoTracker Deep Red**

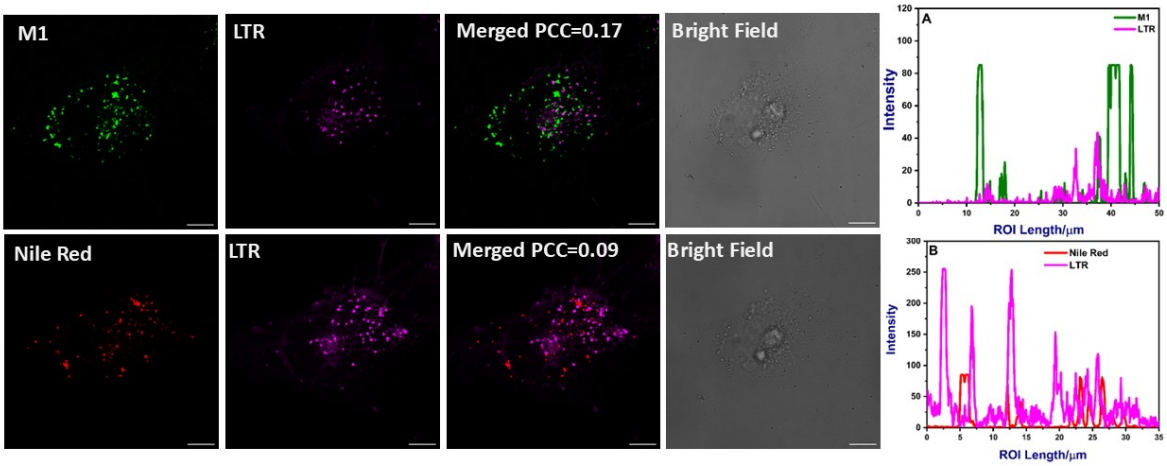


Figure S11: (A) Cells were incubated with **M1** and **LTR** in COS-7 cells, λex = 458 nm; λem = 460-520 nm; λex = 633 nm; λem = 650-700 nm; **(B)** Colocalization of **Nile red** and **LTR** in COS-7 cells, Nile Red λex = 633 nm; λem = 650-700 nm**.** (incubation 10 min at 37 ℃, 5% CO_2, Scale bar=10 μm). Green channel for **M1**, Red channel for Nile Red, Pink channel for **LTR**.

Cellular imaging and colocalization of **M1, LTR** and **Nile Red** together in COS-7 cells**.**

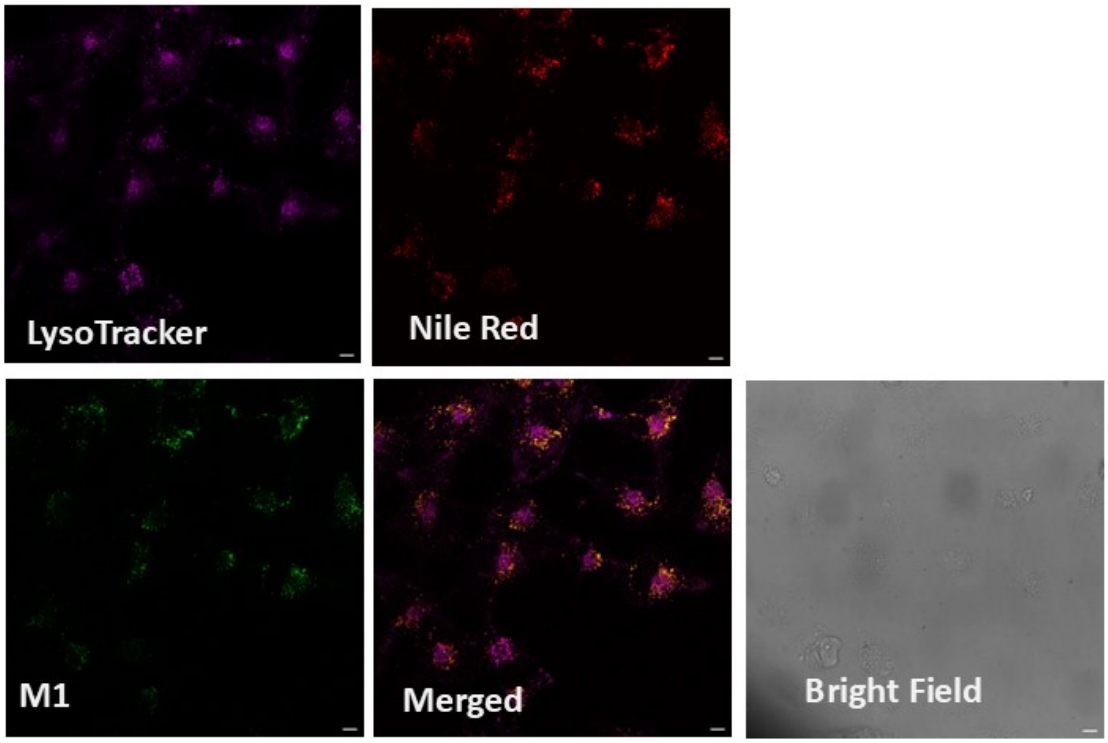


Figure S12: Cells were incubated with **M1**, Nile Red and LTR and incubated with 15 min: **M1**: λ_{ex}: 458 nm; λ_{em}: 460-520 nm. LysoTtracker: λ_{ex}: 633 nm; λ_{em}: 650-700 nm. Nile Red: λ_{ex}: 514nm; λ_{em}: 525-550 nm. (incubation 10 min at 37 °C, 5% CO_2, Scale bar=10 μm). Green channel for **M1**, Red channel for Nile Red, Pink channel for **LTR**.

Tracking lipophagy process using **LTR** and **Nile red**

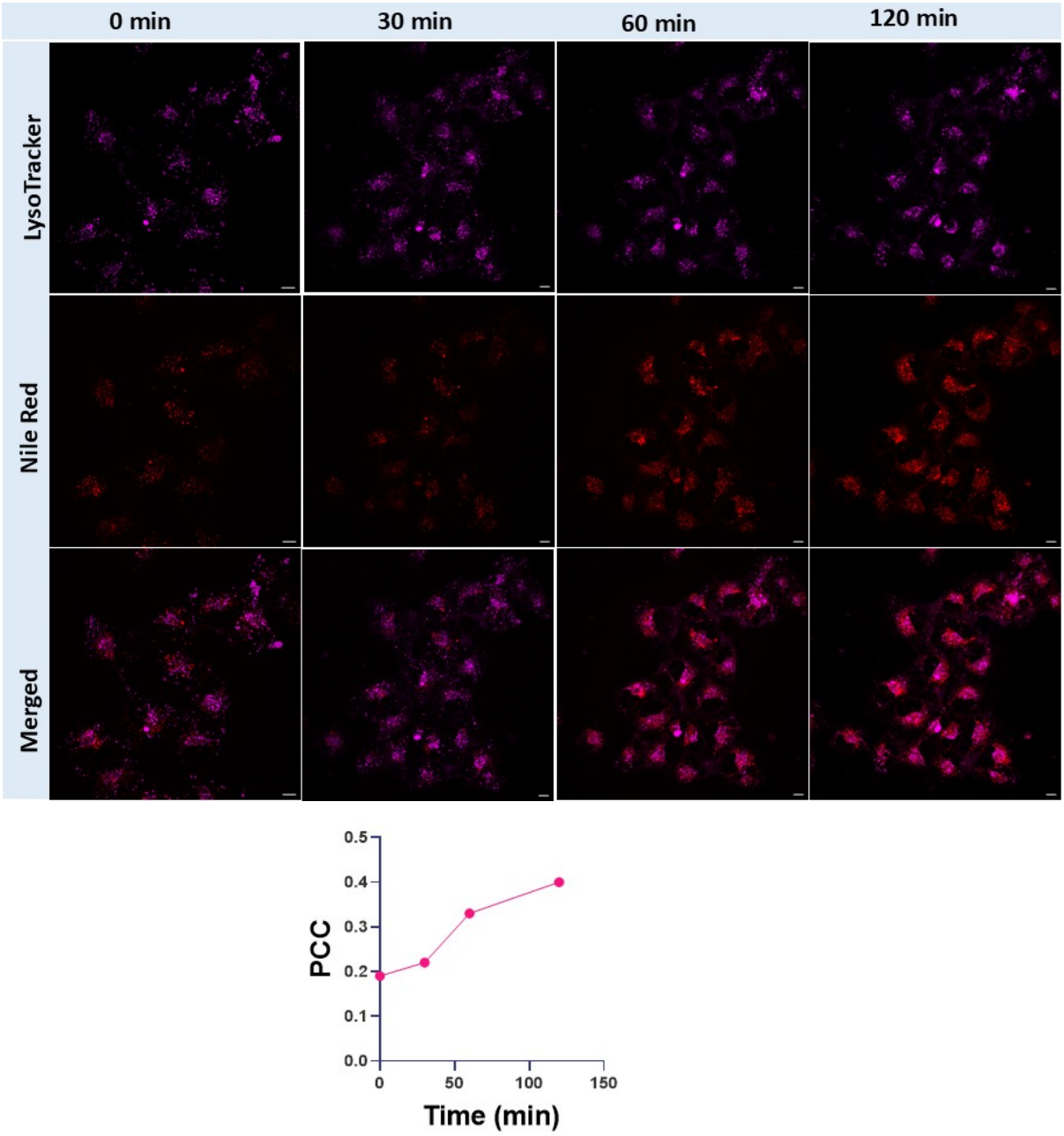


Figure S13: Demonstration of Nile Red in monitoring the lipophagy process. Confocal images lipophagy at different intervals. COS 7 cells stained with **Nile Red** and **LTR** and 50 µM verapamil, which induces the lipophagy. Track the lipophagy at different intervals. **(F)** Enlarged images of the verapamil group for respective time; **M1**: λ_{ex}: 458 nm, λ_{em}: 495-600nm; **LTR,** λ_{ex} 633 nm, λ_{em}: 650-700 nm; Scale bar = 10 µm.

Tracking Mitophagy process using **Nile red** and **Mito Tracker Deep Red**

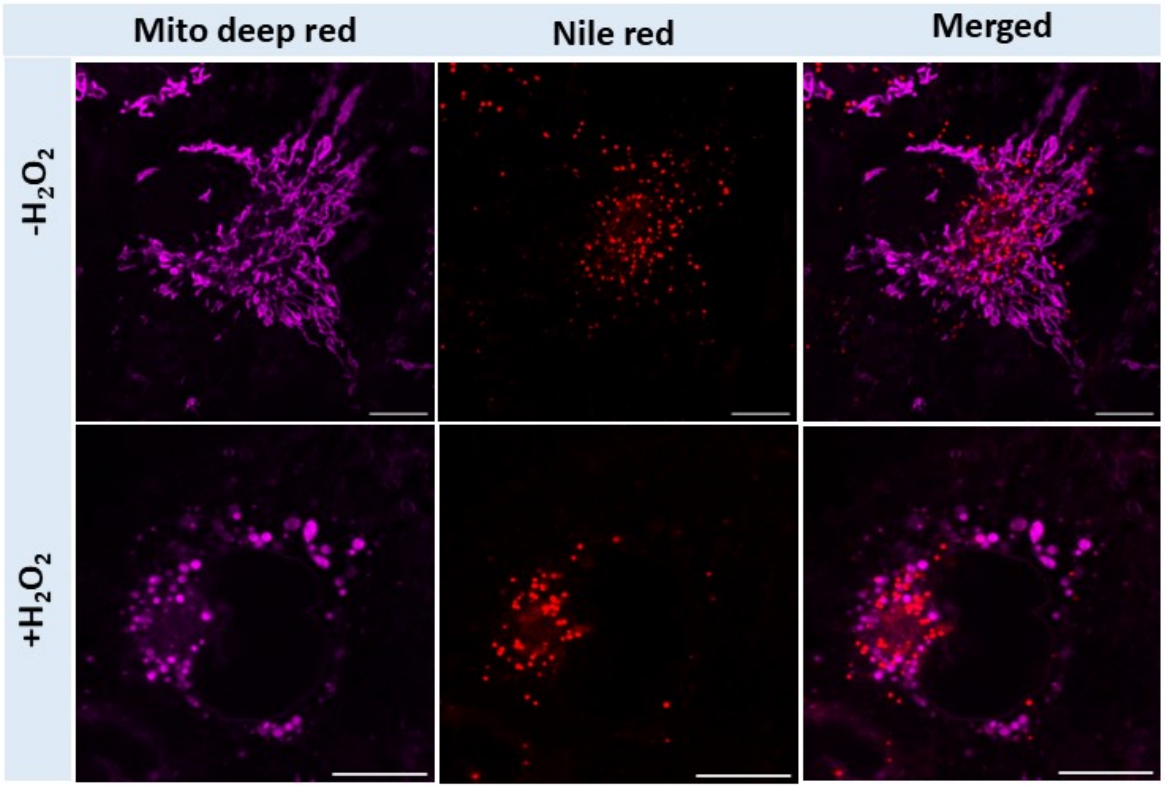


Figure S14: Cellular imaging and colocalization of Nile Red and MTR in COS-7 cells before and after the addition of H_2O_2

Procedure for Photostability of **M1**: COS-7 cells were incubated with **M1** for 10 min, after uptake of M1, confocal imaging was done under continuous light exposure. **M1**= λ_{ex}- 405 nm, λ_{em}- 435-530 nm, video was taken using 512*512 frame with scanning time 400 ns.

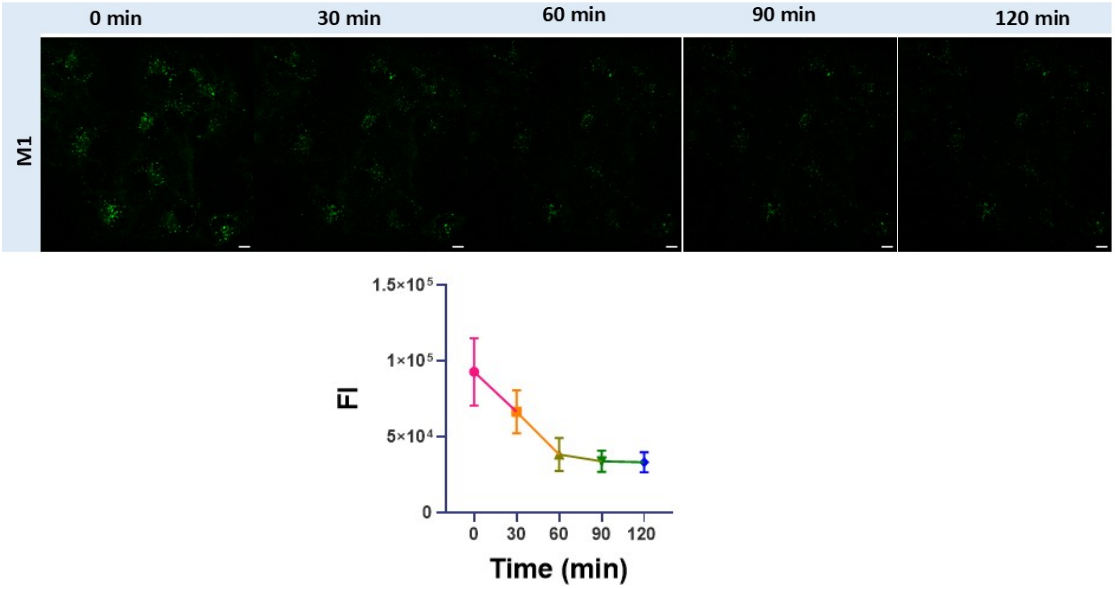
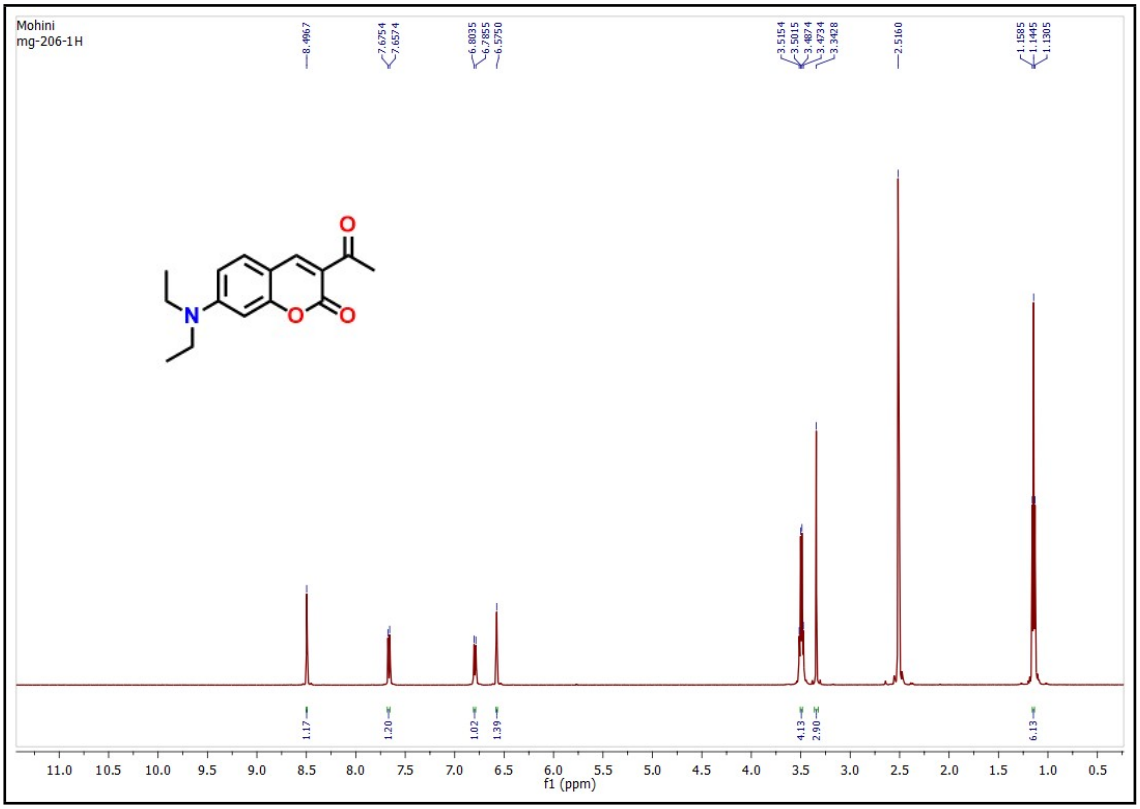
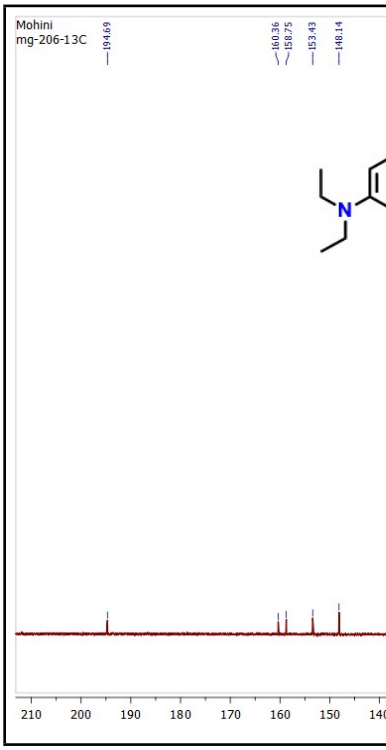


Figure S15: Confocal images of **M1** (2.5 µM) under continuous light exposure, merged images for green channel and bright field for each time interval. (B) The corresponding fluorescence intensity. Scale Bar = 10µm

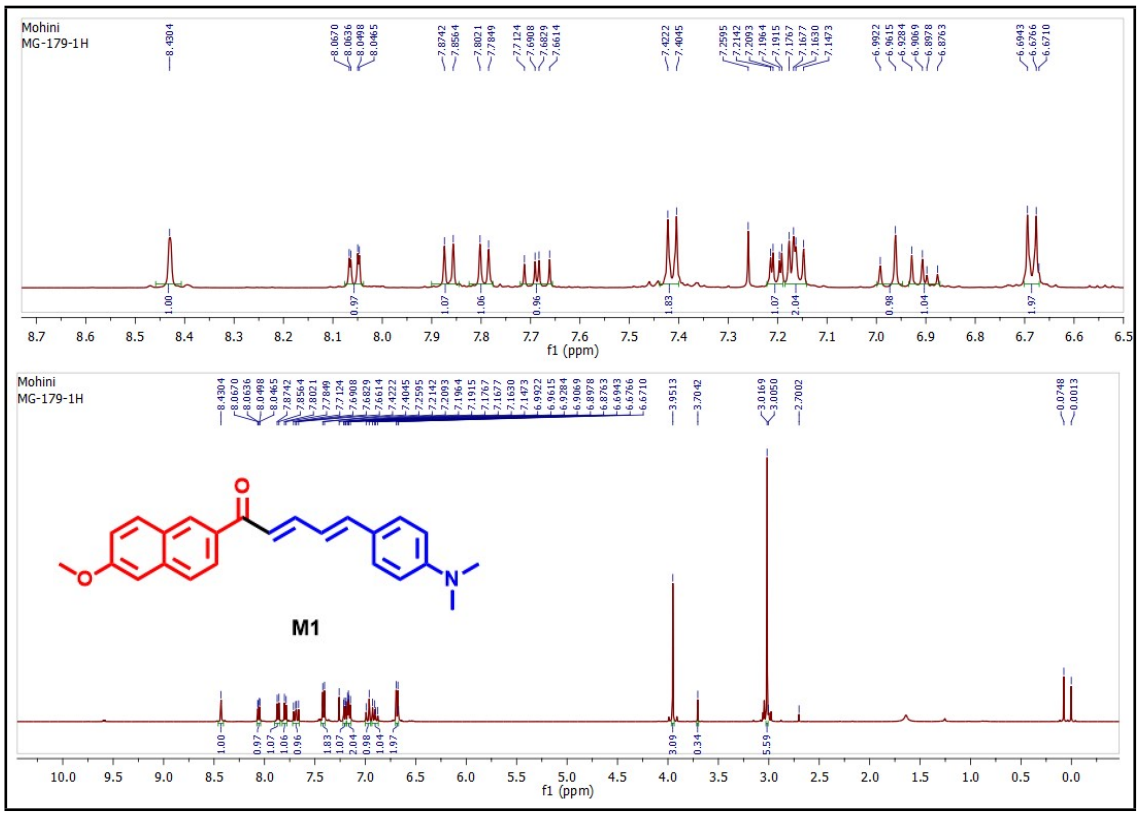
Characterization data: ^{1}H and ^{13}C NMR



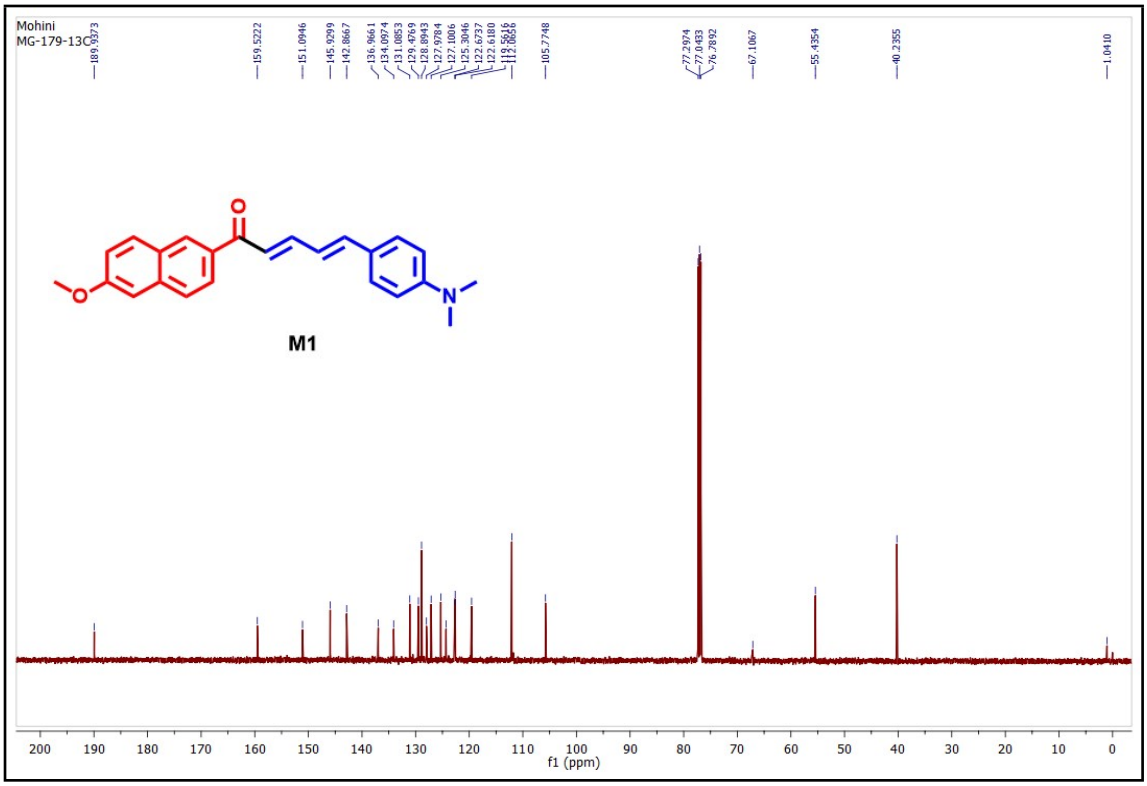
^{1}H NMR of *3-Acetyl-7-(N, N-diethylamino)-2H-chromen-2-one*



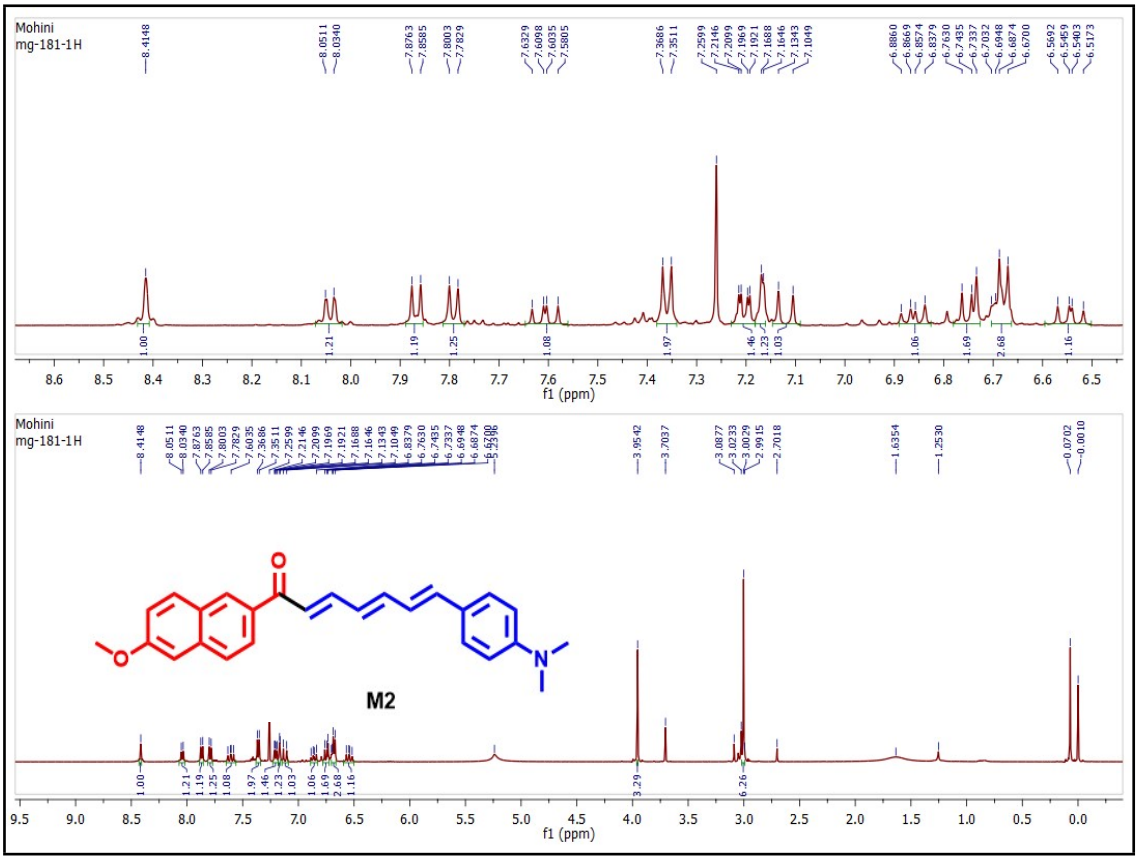
^{13}C NMR of *3-Acetyl-7-(N, N-diethylamino)-2H-chromen-2-one*



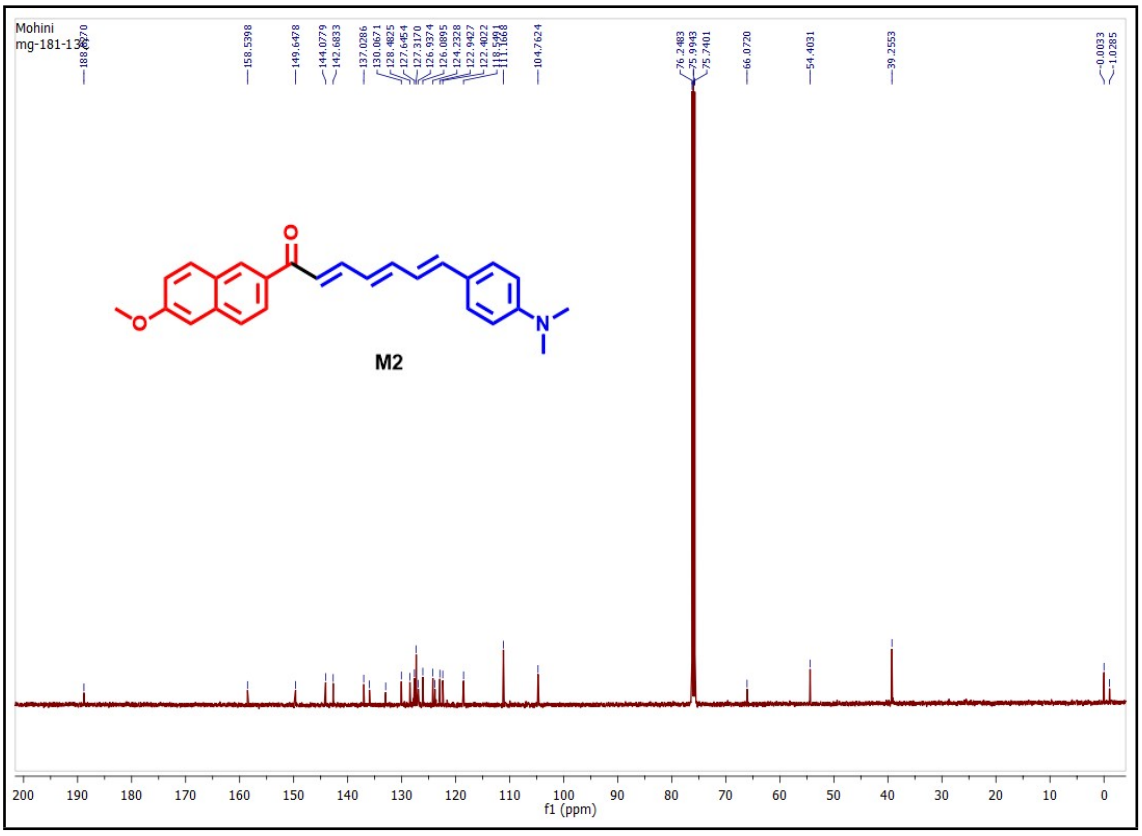
^{1}H NMR of **M1**



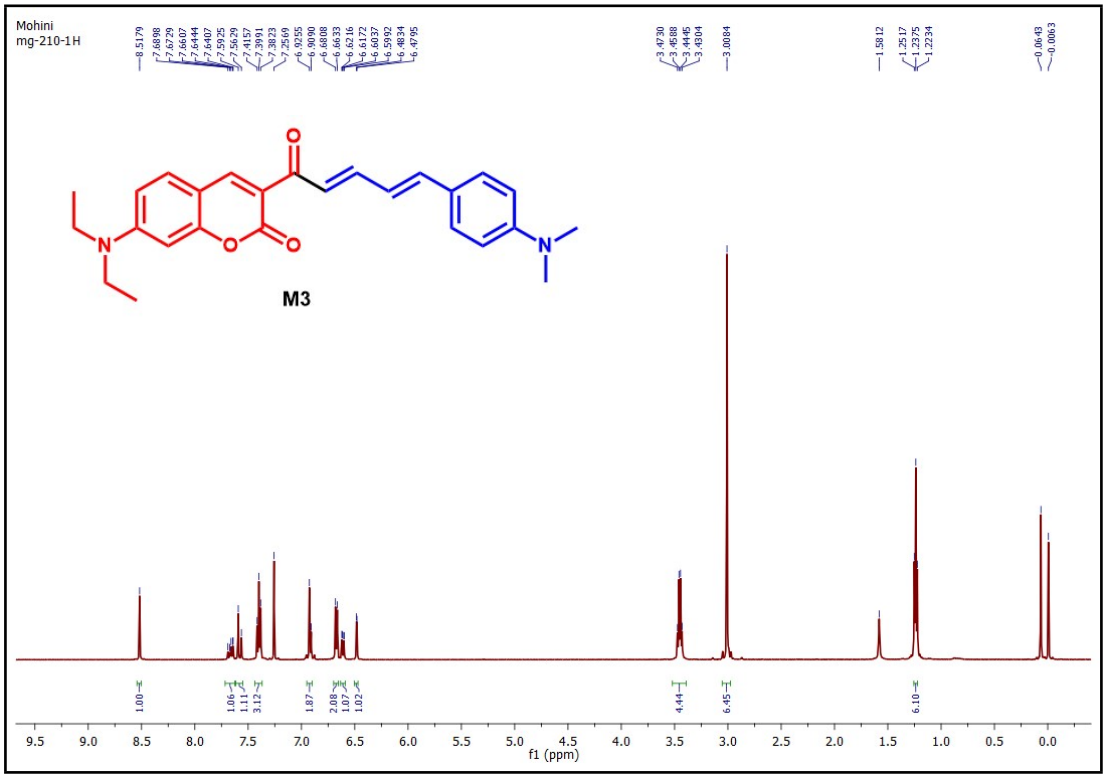
^{13}C NMR of **M1**



^{1}H NMR of **M2**



^{13}C NMR of **M2**



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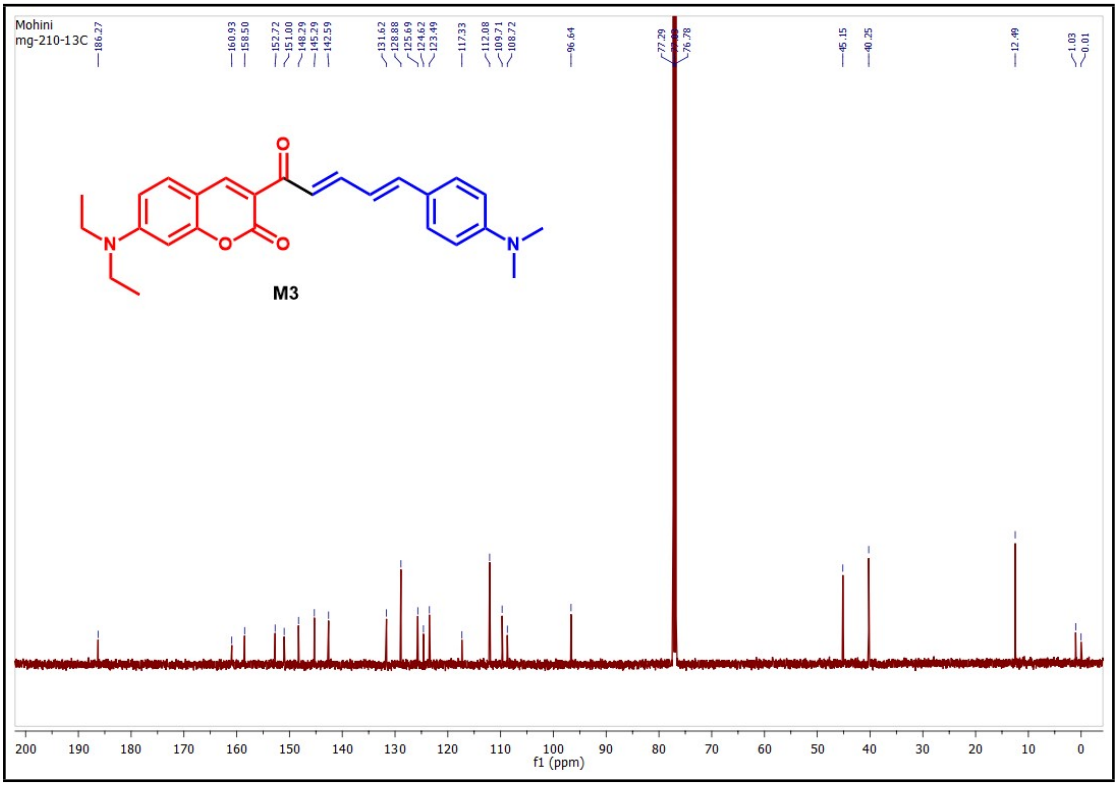
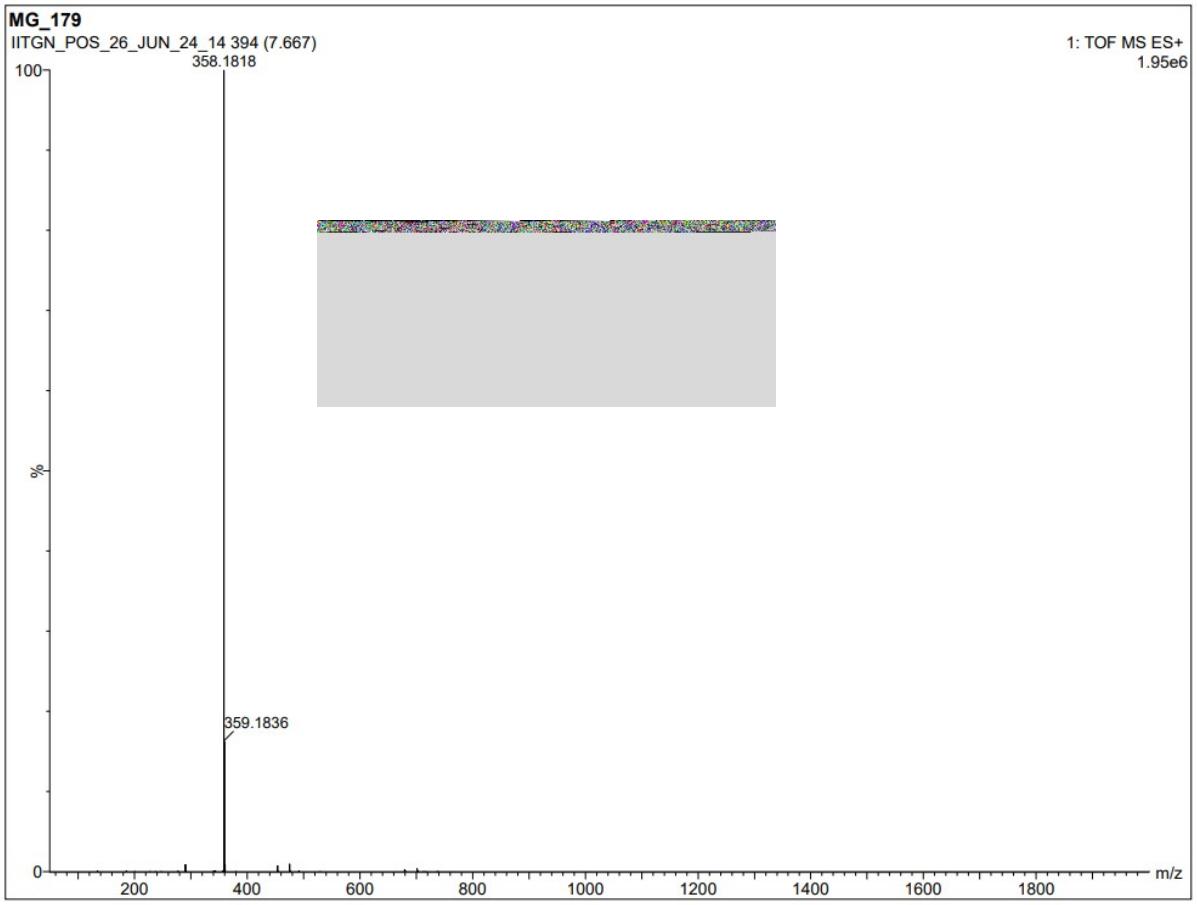
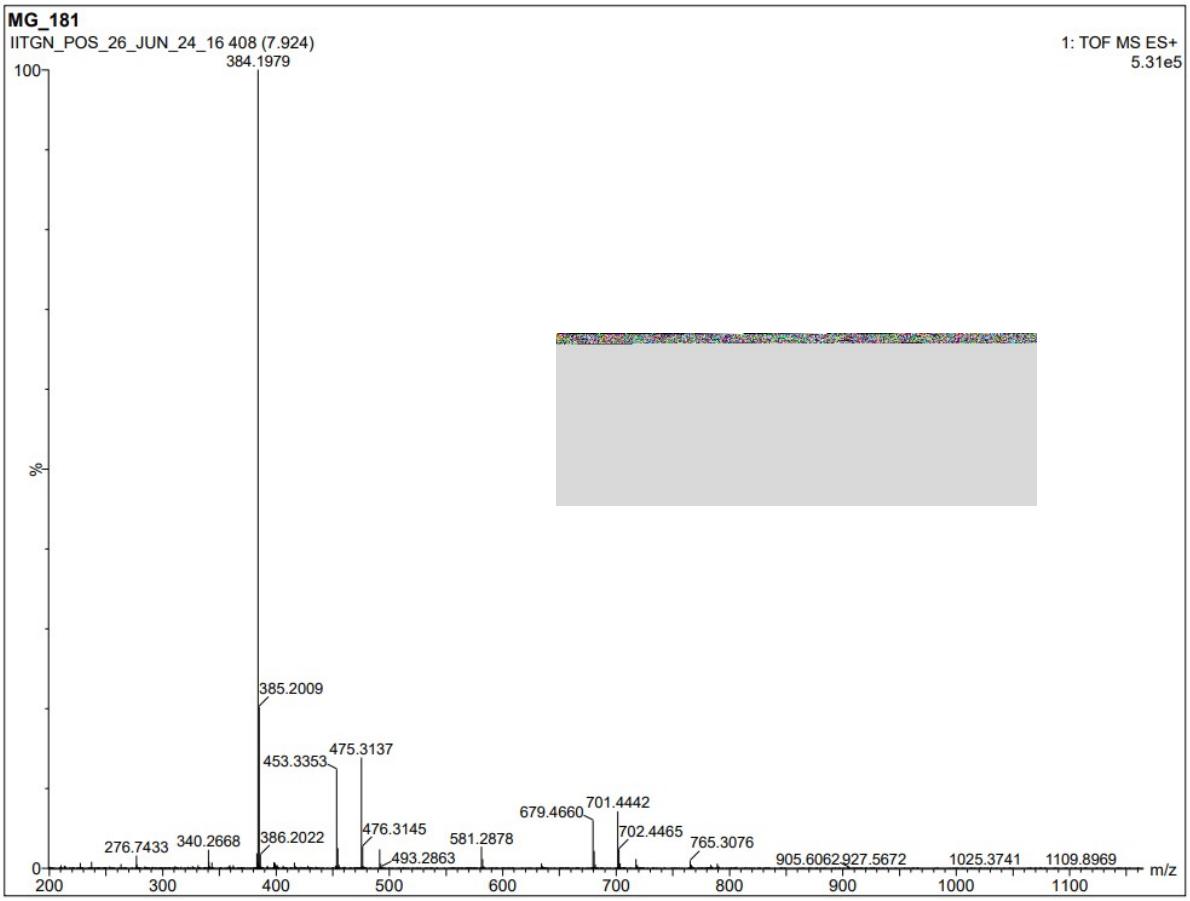


^{13}C NMR of **M3**

Mass spectral data of M1-M3





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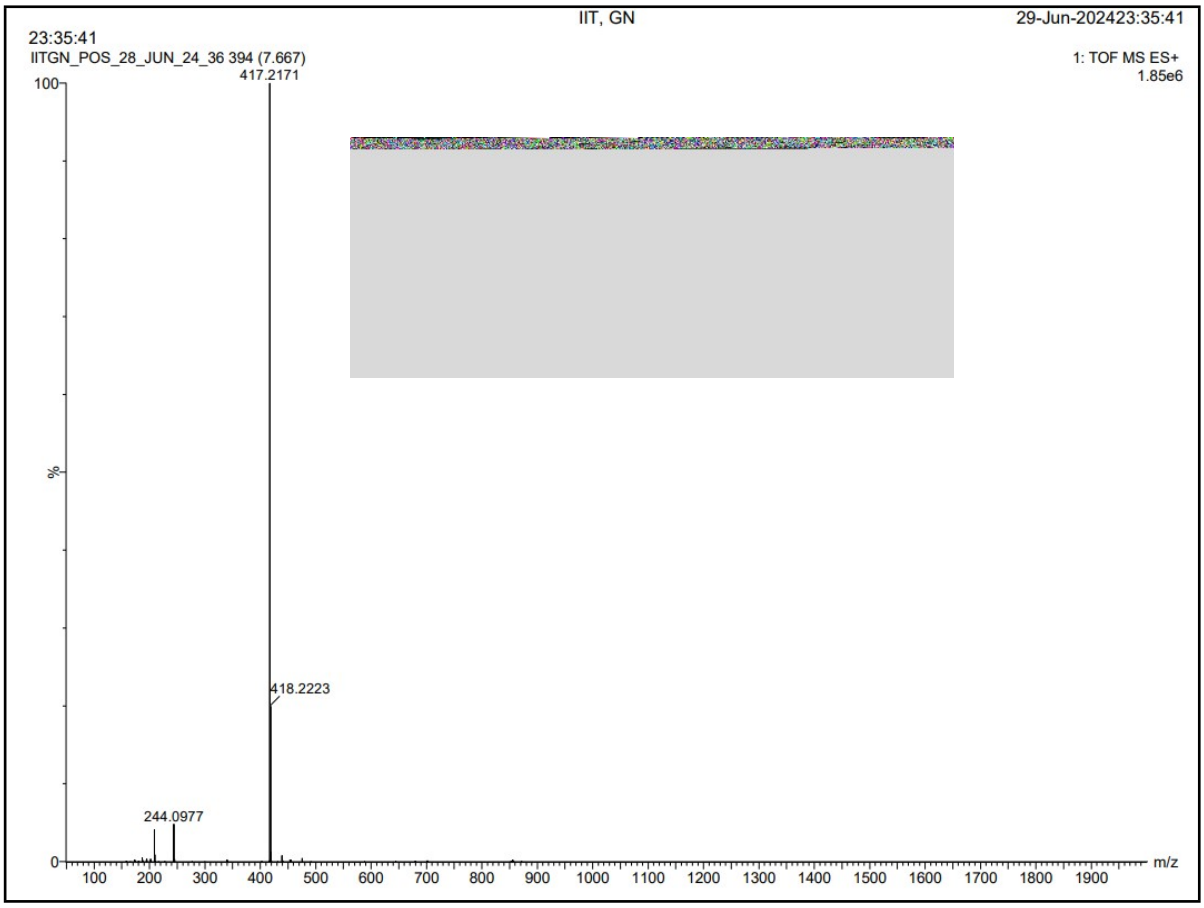
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29-Jun-202423:35:41
23:35:41
IITGN_POS_28_JUN_24_36 394 (7.667)
1: TOF MS ES+
1.85e6
417.2171
418.2223
244.0977
100
%
0
m/z
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900