Supporting information

N-Functionalized fluorophores: Detecting Urinary Albumin and Imaging Lipid Droplets

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Characterization details of M1 to M4

M1*: (Z)-4-(1-cyano-2-(4-(dimethylamino)phenyl)vinyl)-1-tosylpyridin-1-ium chloride.*

^{1}H NMR (500 MHz, DMSO-d6) δ 8.80 (d, *J* = 6.9 Hz, 2H), 8.45 (s, 1H), 8.13 (d, *J* = 7.0 Hz, 2H), 8.06 (d, *J* = 9.1 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 6.92 (d, *J* = 9.2 Hz, 2H), 3.13 (s, 6H), 2.29 (s, 3H). **^{13}C NMR (126 MHz, DMSO-d6)** δ 154.11, 150.44, 143.22, 138.03, 134.16, 128.51, 125.96, 120.93, 120.11, 118.60, 112.47, 40.58, 40.49, 40.41, 40.32, 40.24, 40.15, 40.08, 39.99, 39.82, 39.65, 39.48, 21.25; [M, HRMS=230.1350] (mass found), 230.1339 (actual mass), m/z=0.0011.

M2*: (Z)-4-(1-cyano-2-(4-(dibutylamino)phenyl)vinyl)-1-tosylpyridin-1-ium chloride*

^{1}H NMR (500 MHz, DMSO-d6) δ 8.79 (d, *J* = 6.9 Hz, 2H), 8.42 (s, 2H), 8.13 (d, *J* = 6.9 Hz, 2H), 8.04 (d, *J* = 9.1 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.9 Hz, 2H), 6.89 (d, *J* = 9.2 Hz, 1H), 3.53 – 3.35 (m, 3H), 2.29 (s, 3H), 1.55 (d, *J* = 6.9 Hz, 4H), 1.45 – 1.23 (m, 4H), 0.94 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR (126 MHz, DMSO-d6)** δ 157.32, 155.05, 150.99, 147.61, 142.80, 139.35, 133.27, 130.72, 125.57, 124.44, 123.45, 117.08, 100.08, 55.24, 34.28, 26.00, 24.77, 19.05. [M, HRMS=334.2283] (mass found), 334.2278 (actual mass), m/z=0.0005

M3*: 4-((1Z,3E)-1-cyano-4-(4-(dimethylamino)phenyl)buta-1,3-dien-1-yl)-1-tosylpyridin-1-ium chloride.*

^{1}H NMR (500 MHz, DMSO-d6) δ 8.79 (d, *J* = 6.7 Hz, 2H), 8.51 (d, *J* = 11.5 Hz, 1H), 8.04 (d, *J* = 6.7 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 14.7 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.19 – 7.13 (m, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 6.80 (d, *J* = 8.9 Hz, 2H), 3.07 (s, 6H), 2.29 (s, 3H). **^{13}C NMR (126 MHz, DMSO-d6)** δ 153.13, 152.80, 151.42, 146.27, 143.56, 138.03, 131.75, 128.52, 125.97, 122.72, 120.98, 119.21, 116.54, 112.63, 101.72, 40.58, 40.49, 40.42, 40.33, 40.25, 40.16, 40.08, 39.99, 39.82, 39.66, 39.49, 21. [M, HRMS=274.1493] (mass found), 274.1495 (actual mass), m/z=0.0002

M4: (Z)-4-(1-cyano-2-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)vinyl)-1-tosylpyridin-1-ium chloride

^{1}H NMR (500 MHz, DMSO-d6) δ 8.85 (d, *J* = 6.7 Hz, 2H), 8.81 (s, 1H), 8.29 (s, 1H), 8.11 (d, *J* = 6.4 Hz, 2H), 7.66 (d, *J* = 9.1 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.9 Hz, 2H), 6.87 (dd, *J* = 9.1, 2.3 Hz,1H), 6.68 (d, *J* = 2.1 Hz, 1H), 3.55 (d, *J* = 7.1 Hz, 4H), 2.29 (s, 3H), 1.17 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR (126 MHz, DMSO-d6)** δ 160.62, 157.85, 153.78, 146.24, 145.02, 144.30, 138.05, 132.69, 128.52, 125.96, 121.80, 117.17, 111.55, 111.29, 108.58, 97.17, 45.15, 40.57, 40.48, 40.41, 40.32, 40.24, 40.15, 40.07, 39.98, 39.82, 39.65, 39.48, 21.25, 12.91. [M, HRMS=346.1558] (mass found), 346.1539 (actual mass), m/z=0.0019.

S1: (A-C) Absorption spectra of **M1-M3** in different solvents

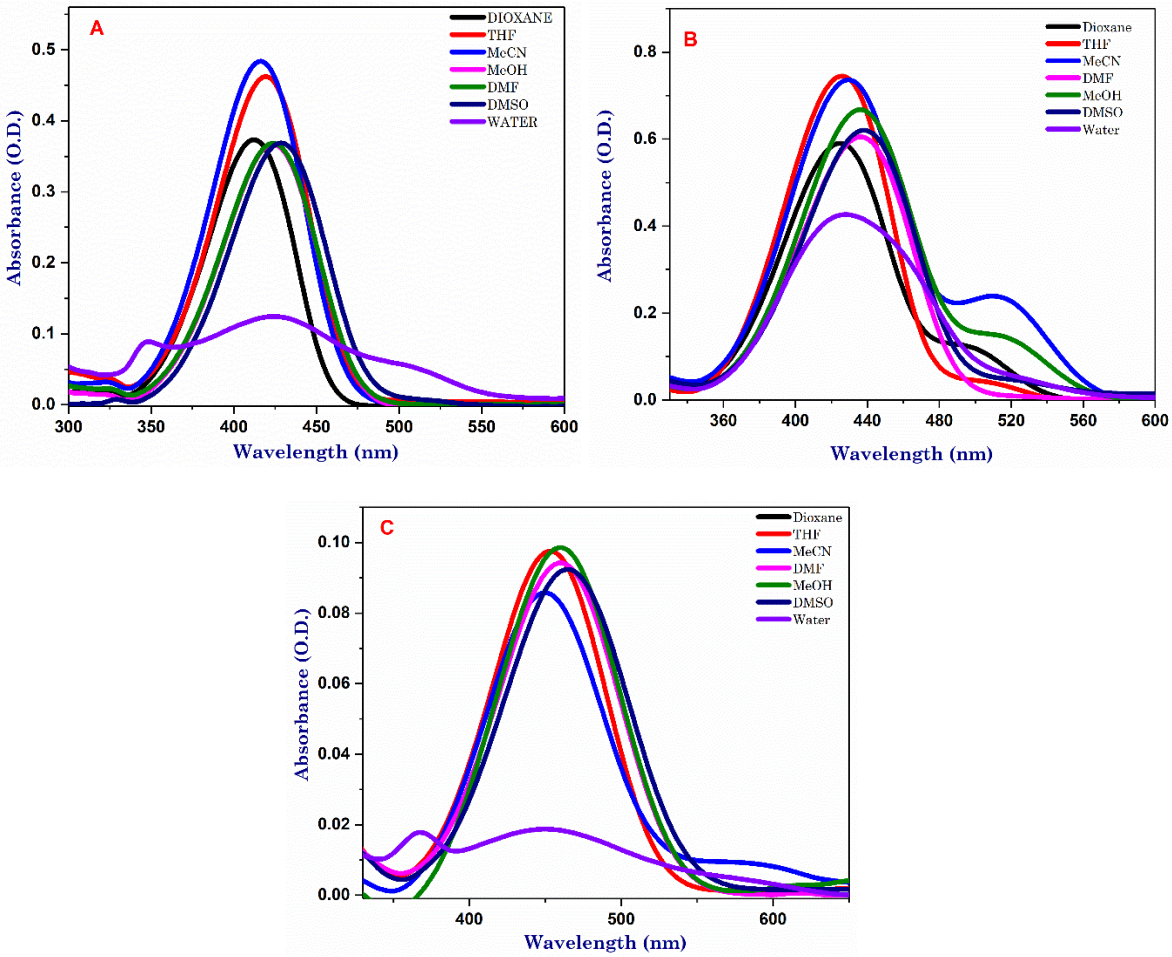


Fig S2: **(D-F)** The emission spectra of **M1-M3** in different solvents.

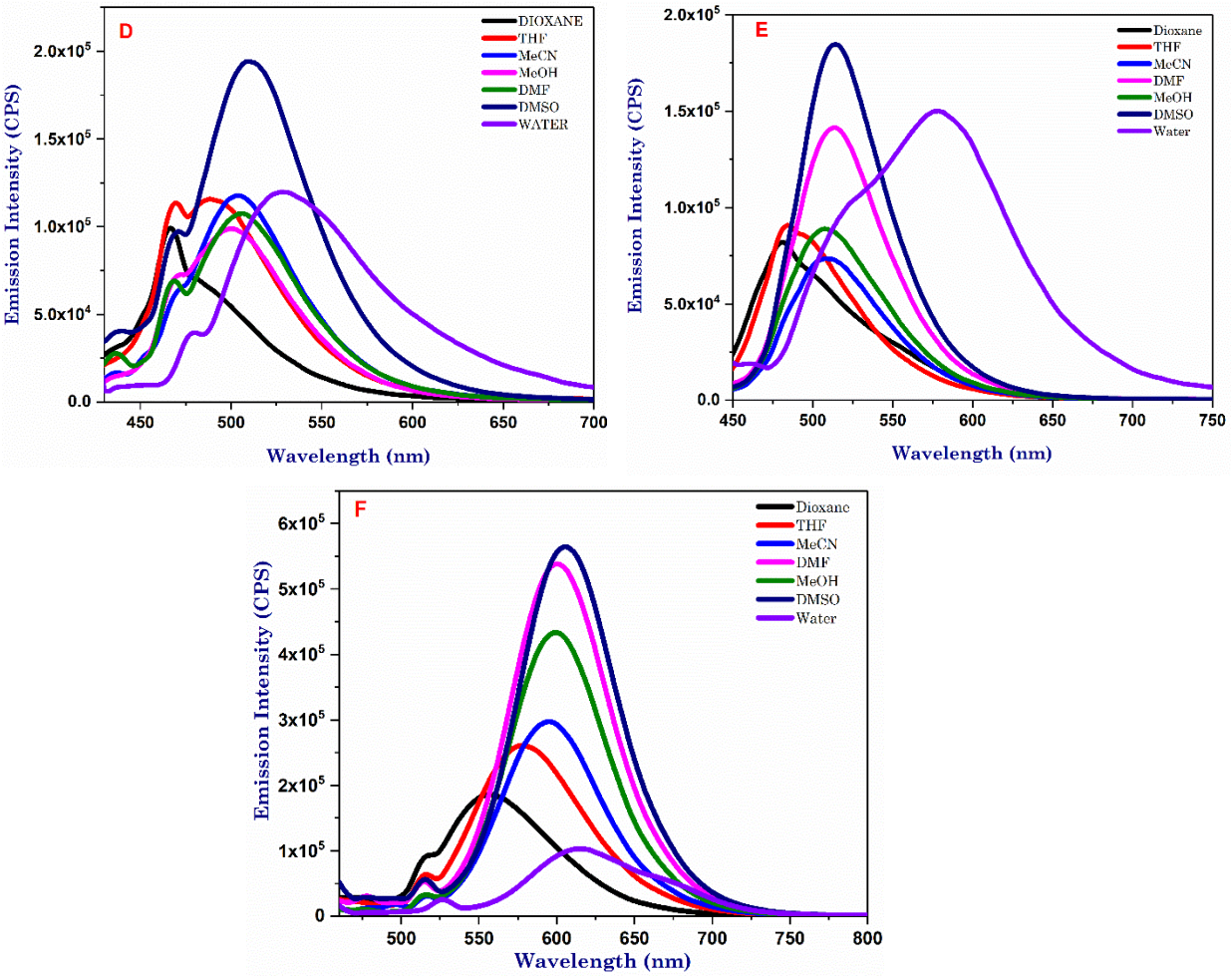


Fig S3: BSA interaction study: The emission spectral changes of the **(A-C)** showing BSA interaction with **M1**, **M2** and **M4** respectively.

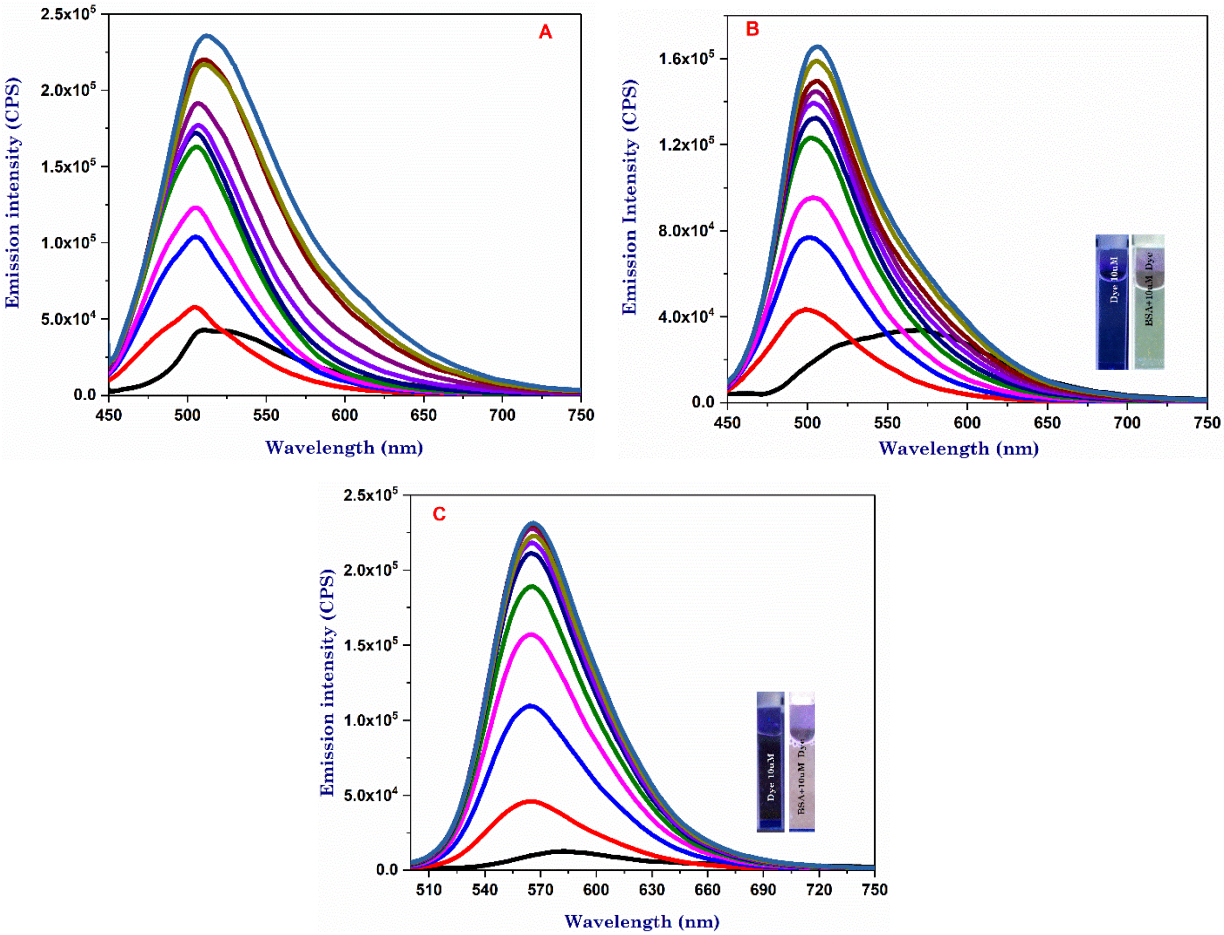
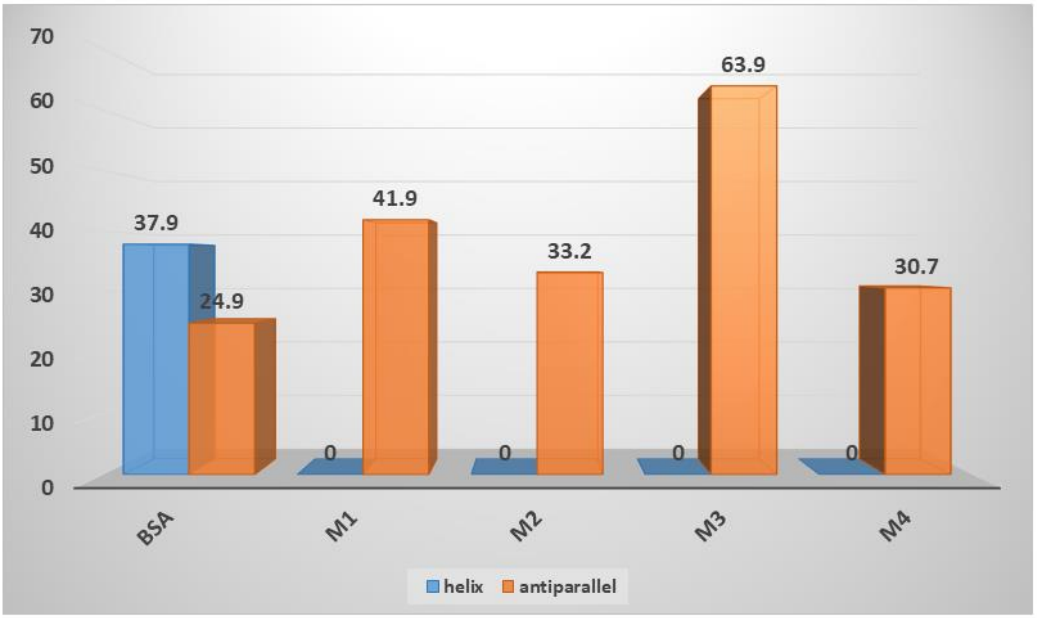


Table S1: Protein conformational changes upon binding of the ligands

	BSA	**M1**	**M2**	**M3**	**M4**
α-Helix	37.9	0	0	0	0
Antiparallel β-sheets	24.9	41.9	33.2	63.9	30.7



FigS4: Isothermal titration profile **M1, M2 & M4** respectively**.**

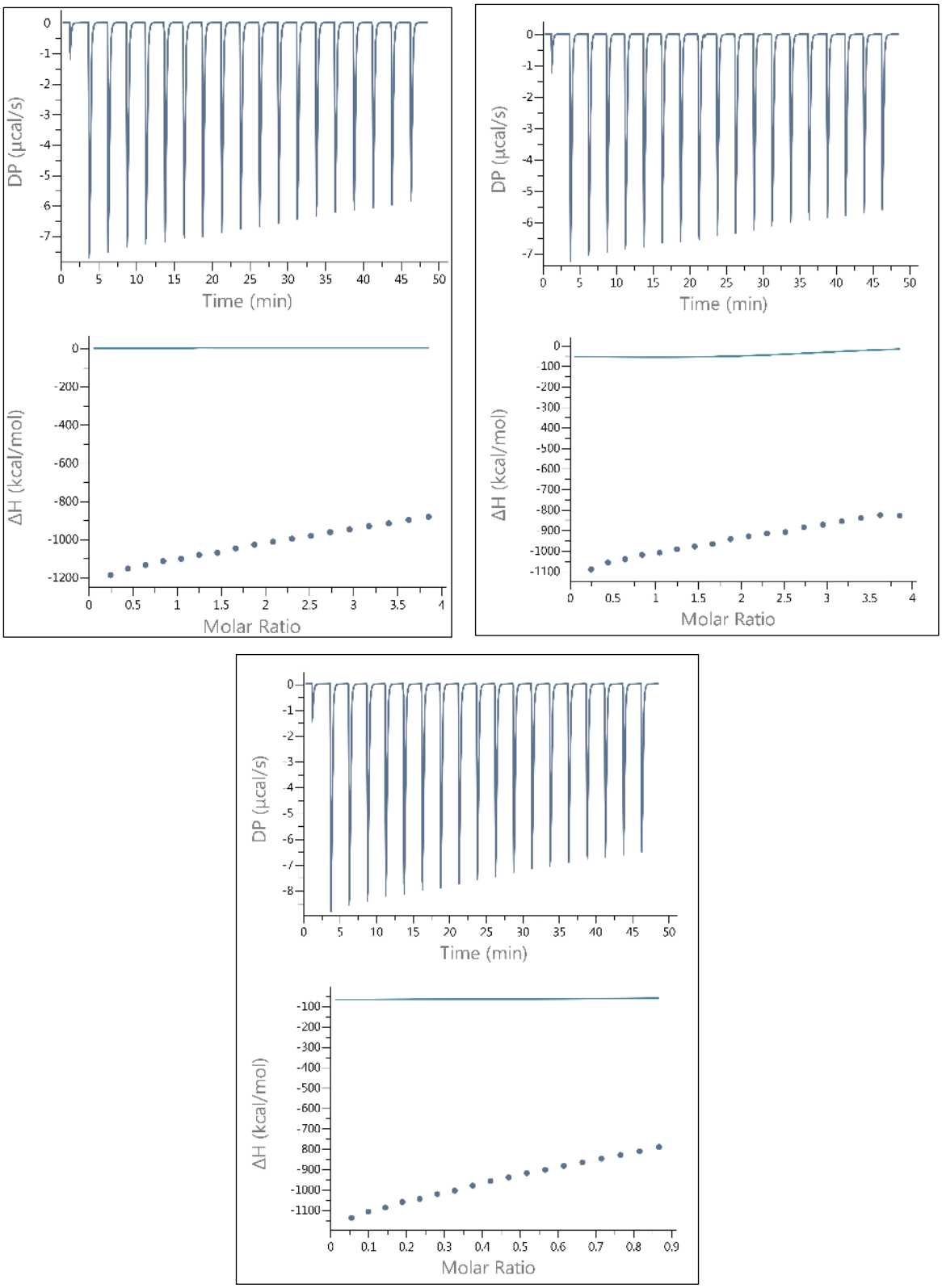
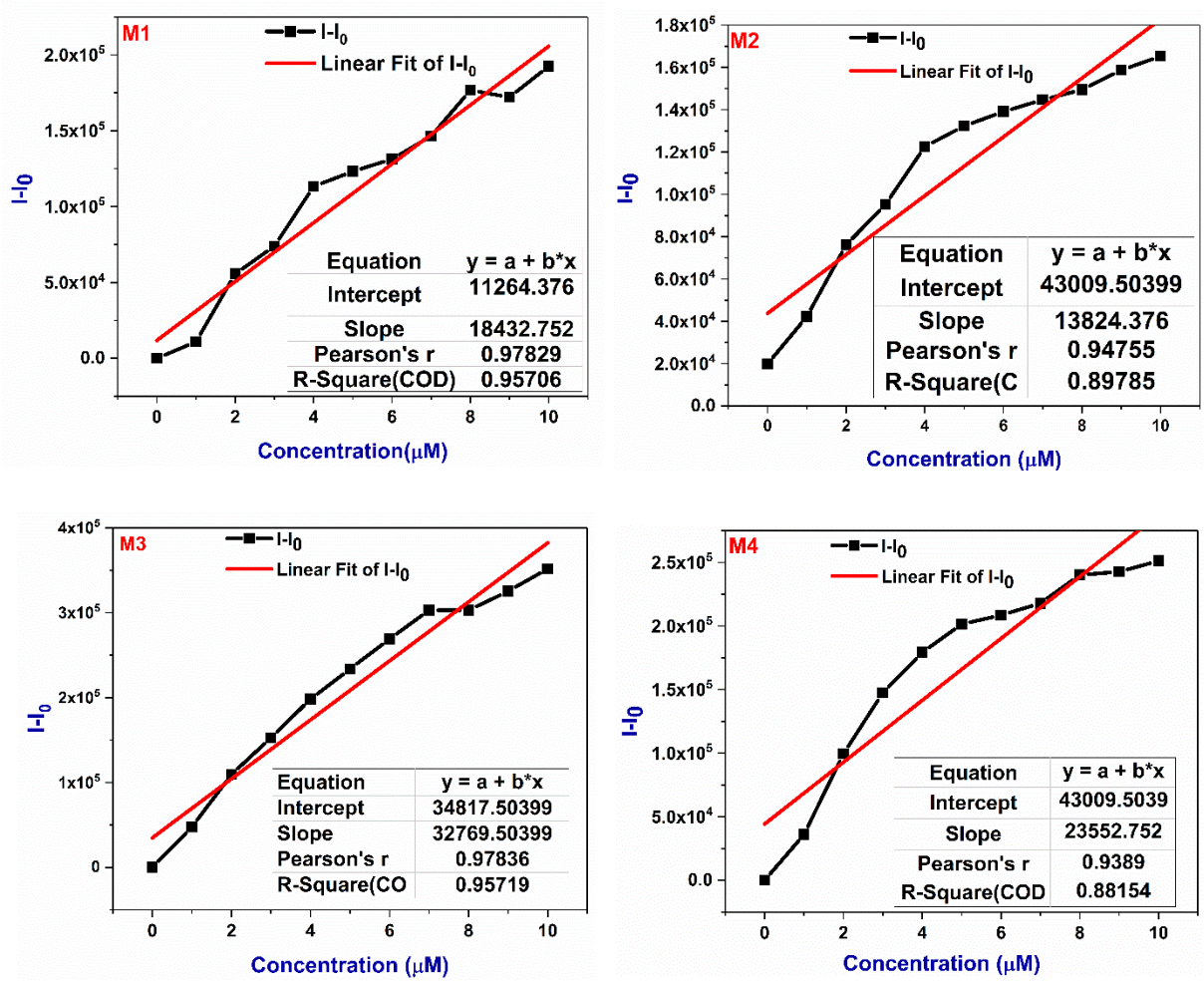


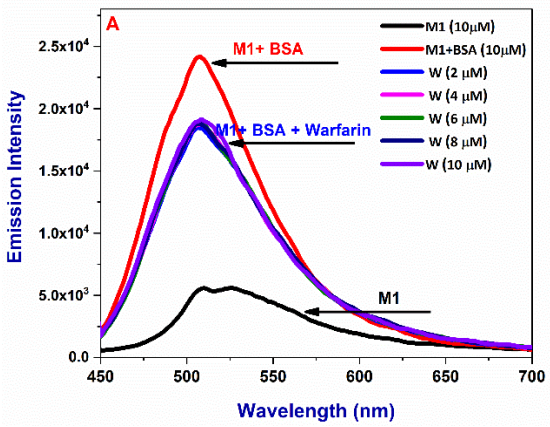
Table S2:

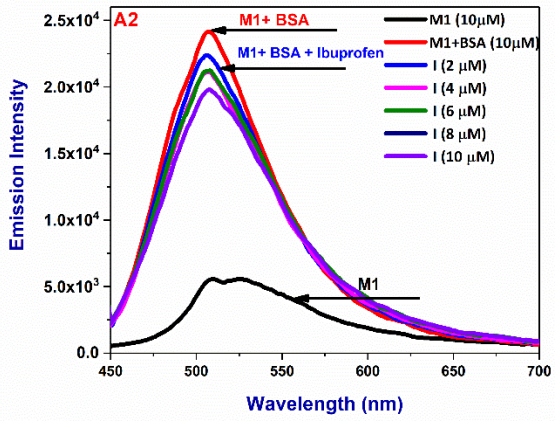
	M1	**M2**	**M3**	**M4**
Kb1	$7.88e^{-07}$	$5.58e^{-07}$	$1.08e^{-04}$	$3.38e^{-06}$
ΔG1	-8.33	-8.53	-5.41	-7.46

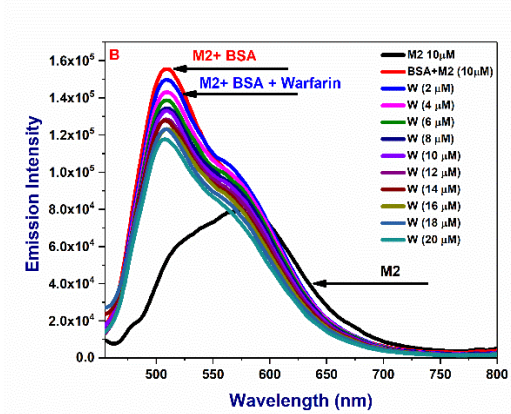
Fig S5: The limit of detection plots for the **M1 to M4**

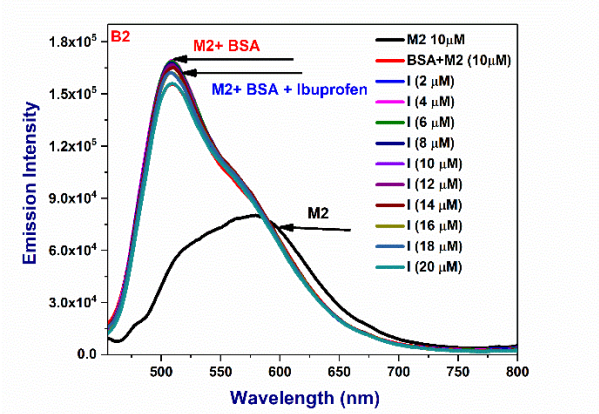


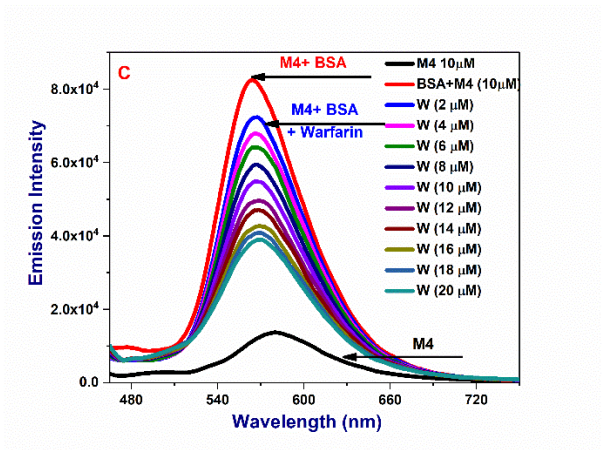
FigS6: Competitive assay of **M1** with Warfarin (A) and Ibuprofen (A2) and **M2** with Warfarin (B) and Ibuprofen (B2) **M4** (Warfarin C and Ibuprofen C2).











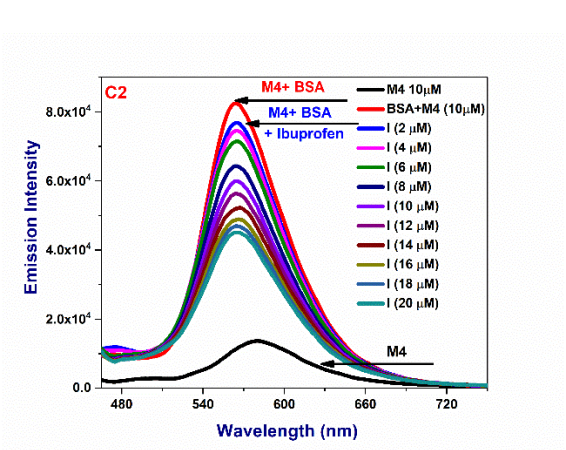


Fig S7: Molecular docking: Ligand interaction diagrams of **M3 and M4**

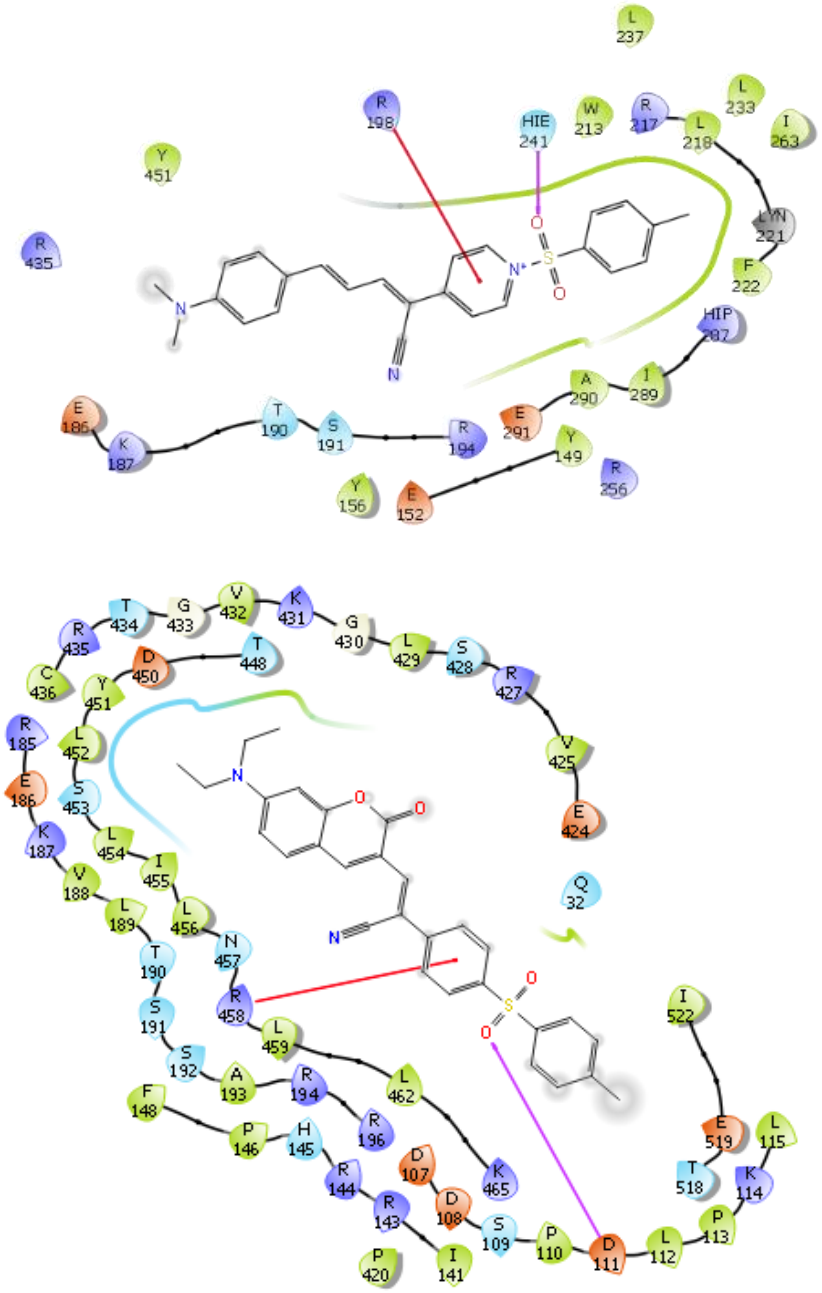


Fig S8: BSA sensing in artificial urine using **M1- M2, M3**

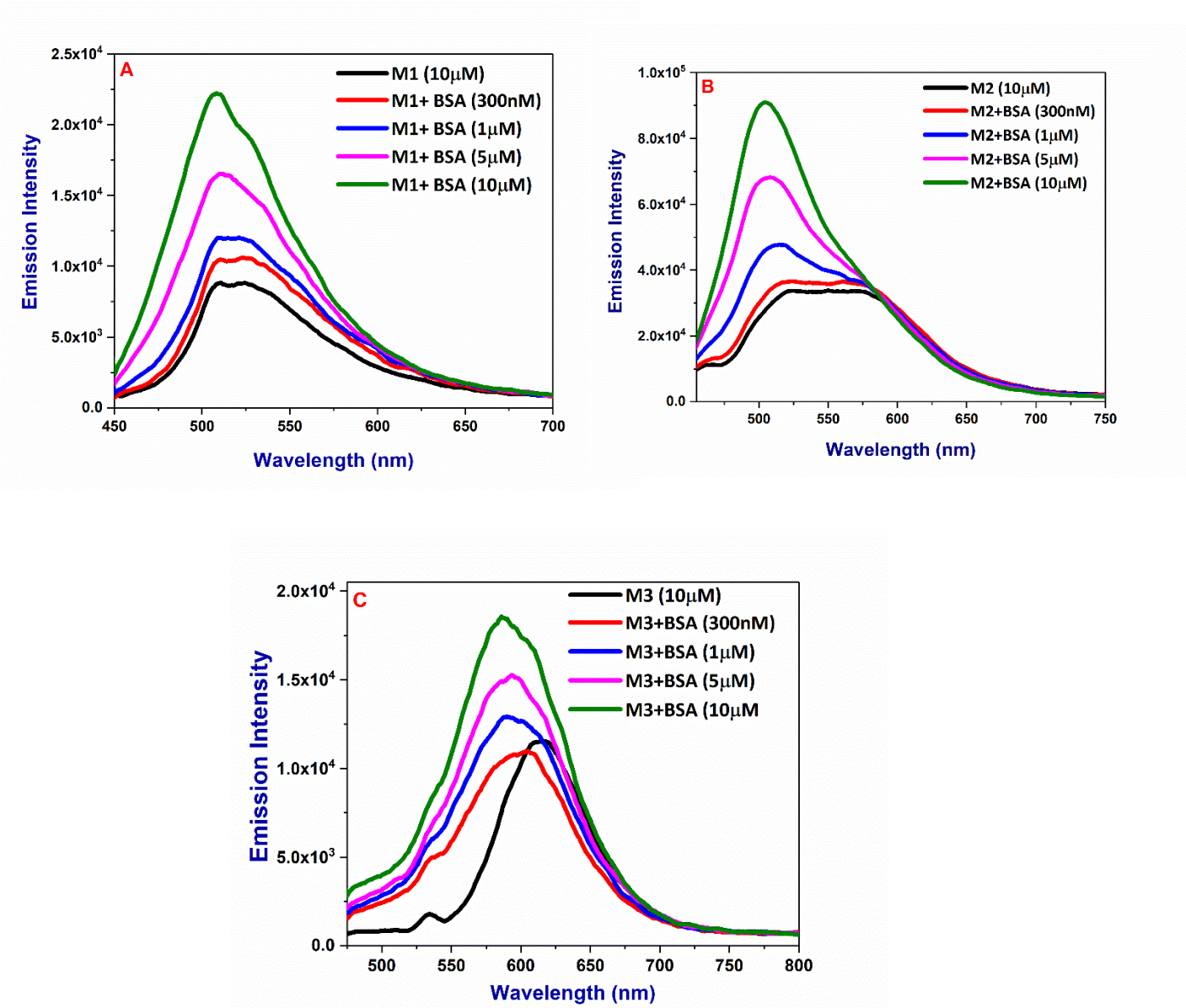


Fig S9: Cellular imaging with **M2** in Cos-7 cells. (Scale: 10 μm): A: **M2**-alone, B: Nile red (20 nM); C: merged image; D: **M2** with bright field images of the droplets.

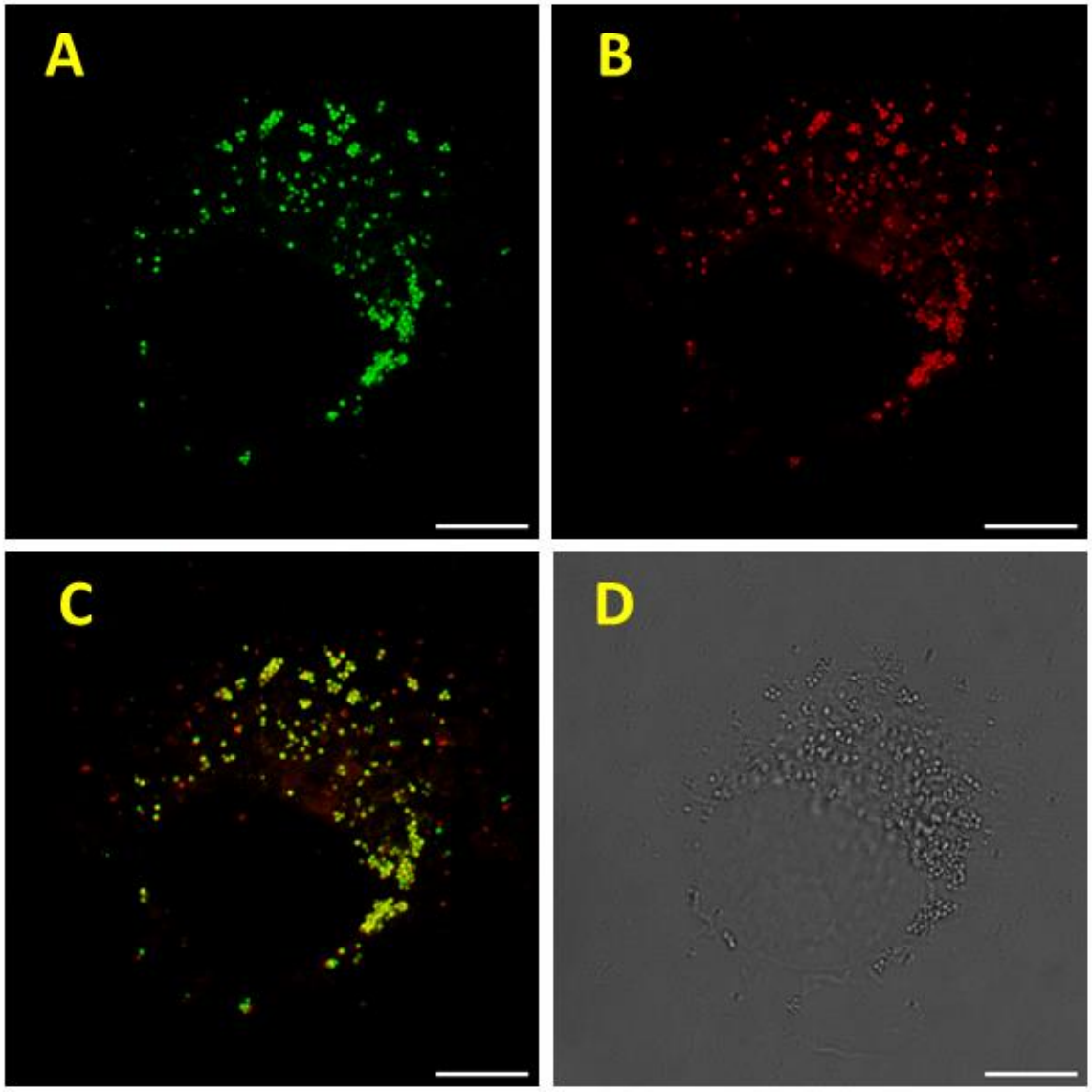


Fig S10: Cellular imaging of **M3** in Cos-7 cells A: **M3** alone, B **M3** overlapped with bright field image of the cells: Overlap noted with lipid droplets is weak and the dye is scattered at other places: Scale (10 μm).

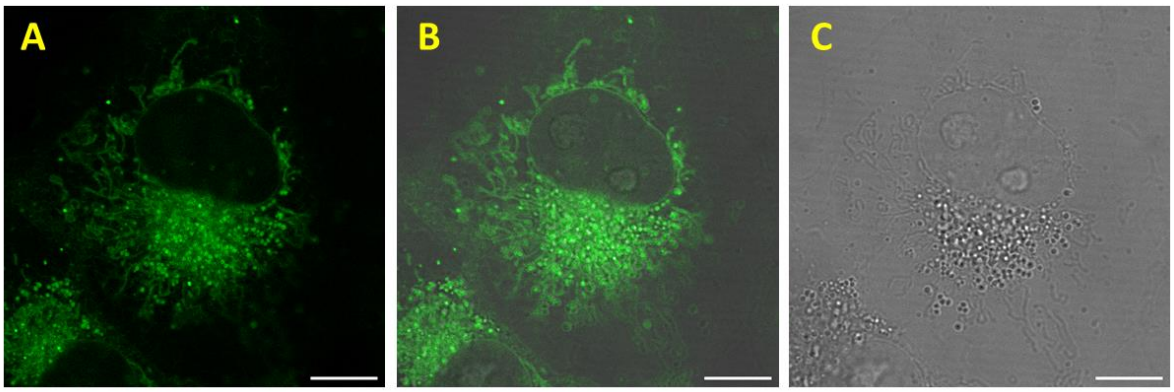
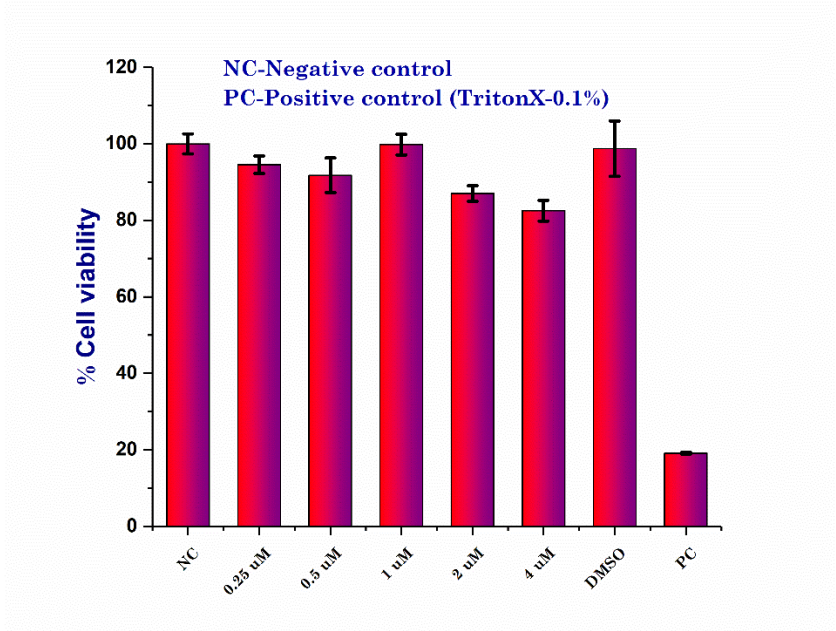
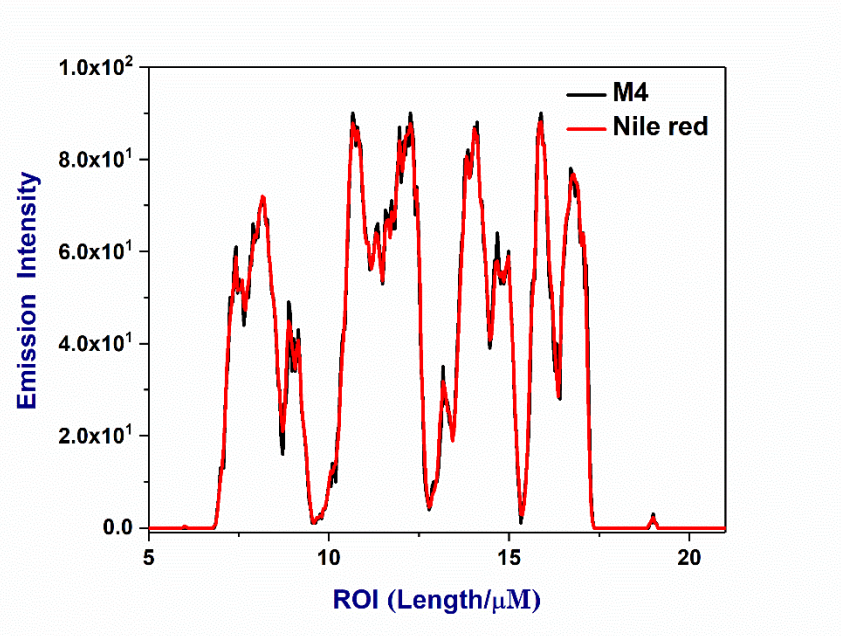


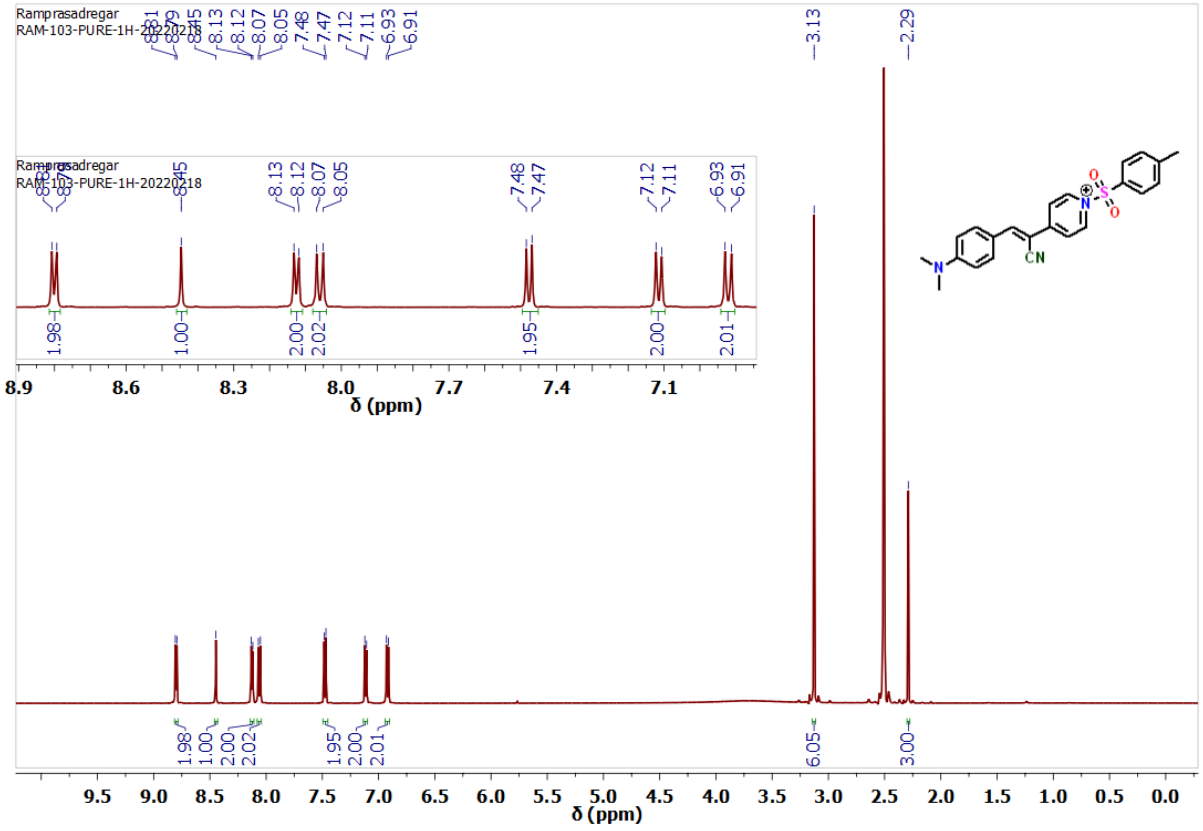
Fig S11: MTT Assay of **M4** and colocalization line profile (below) plot of **M4** with Nile Red.

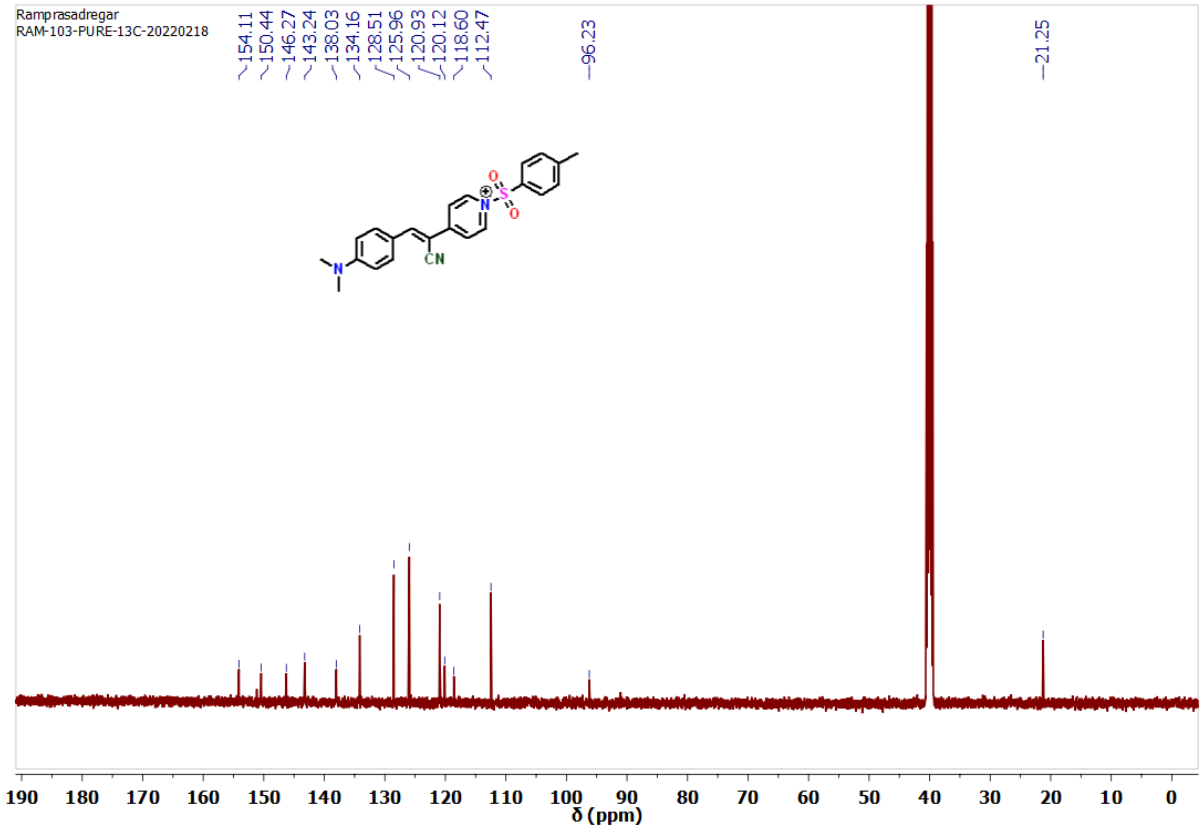


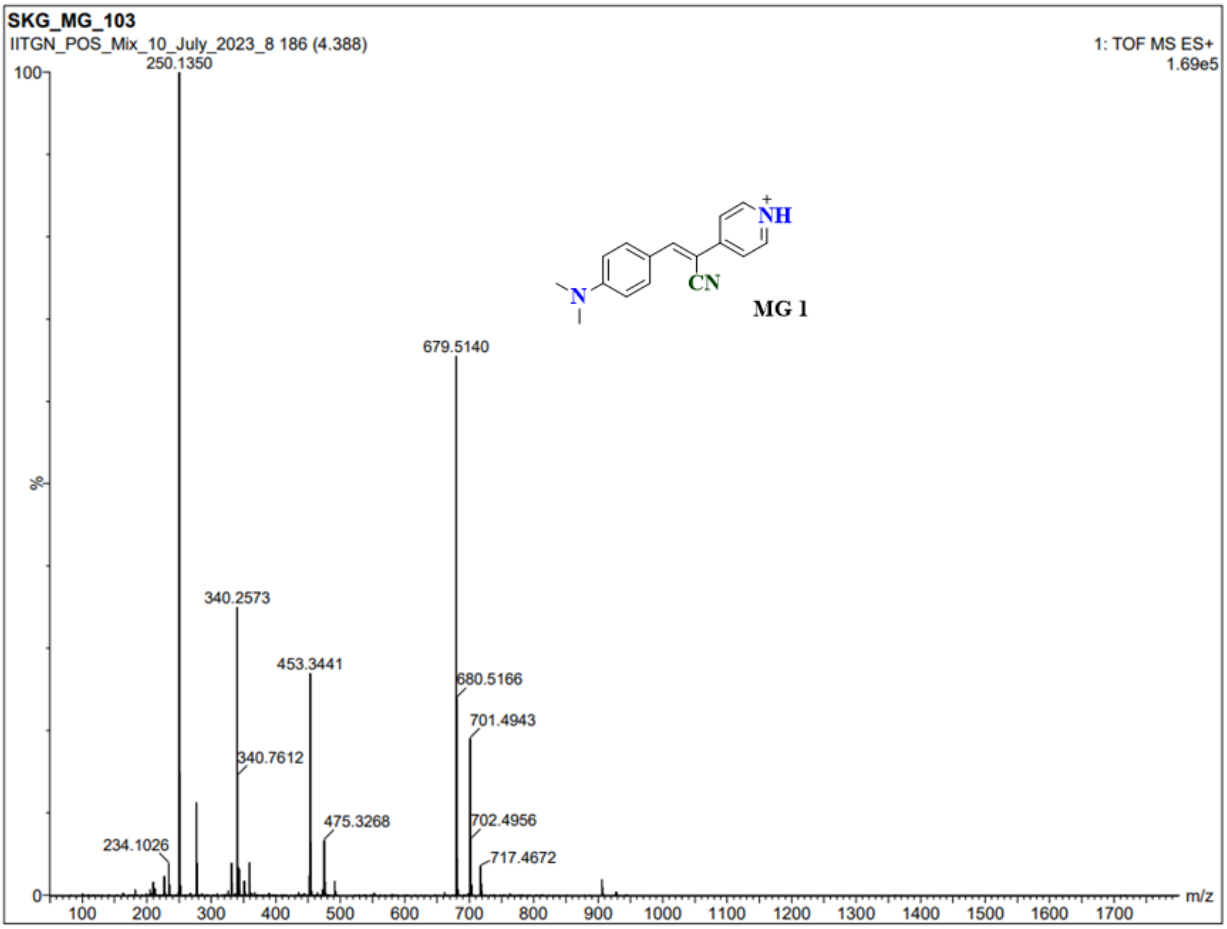


Characterization Spectral data

^{1}H, ^{13}C NMR and Mass data of compound M1- M4:



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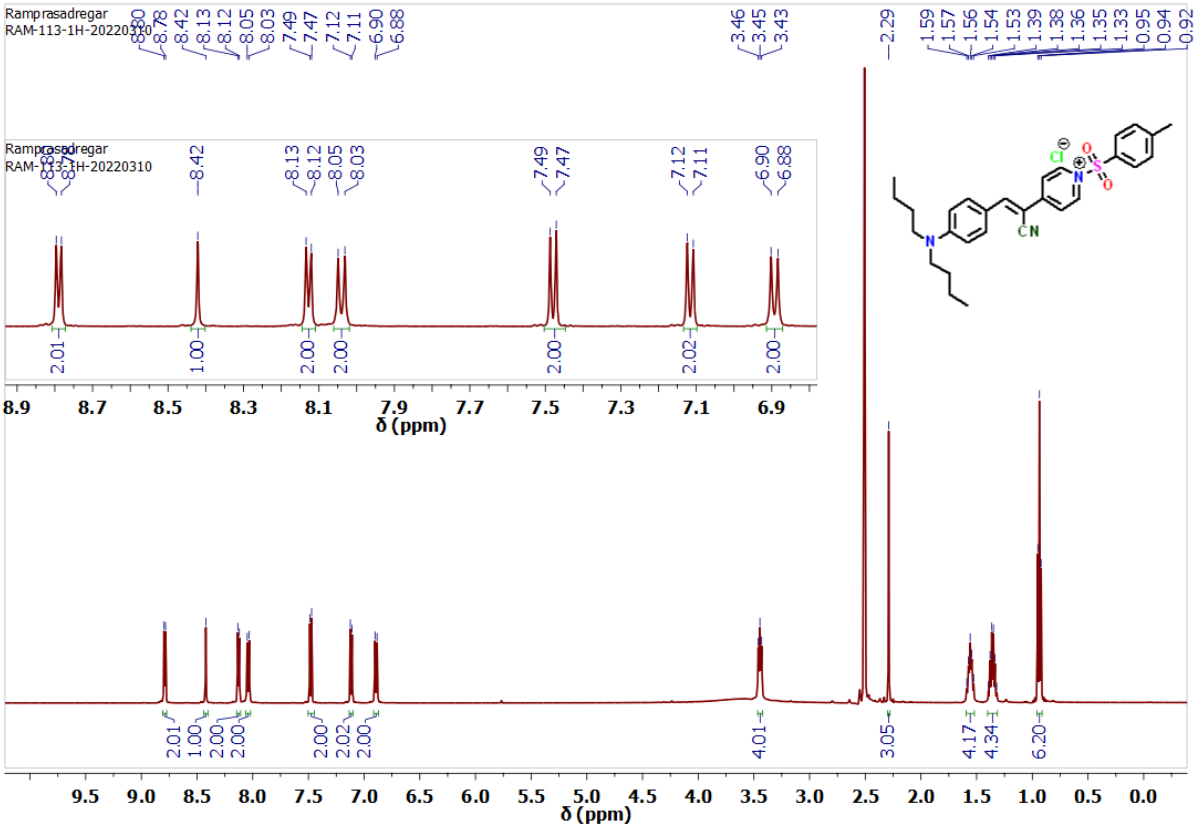
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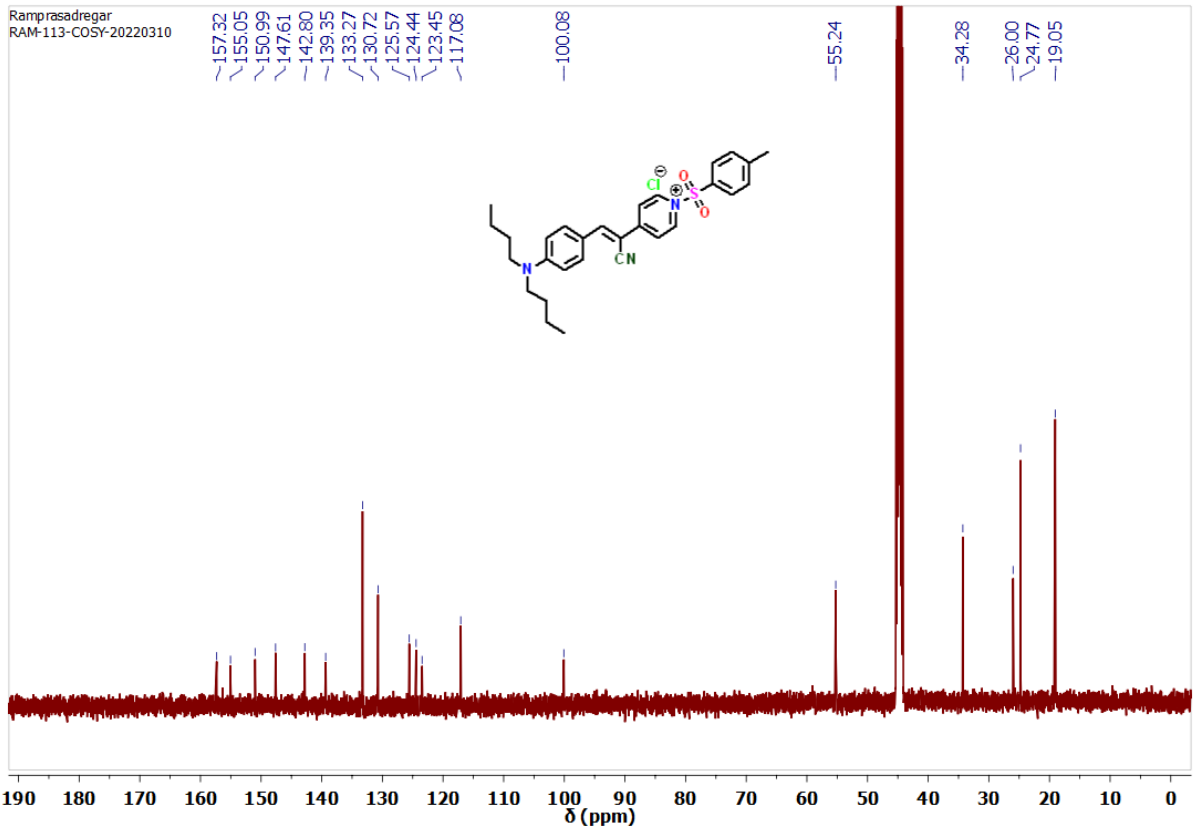
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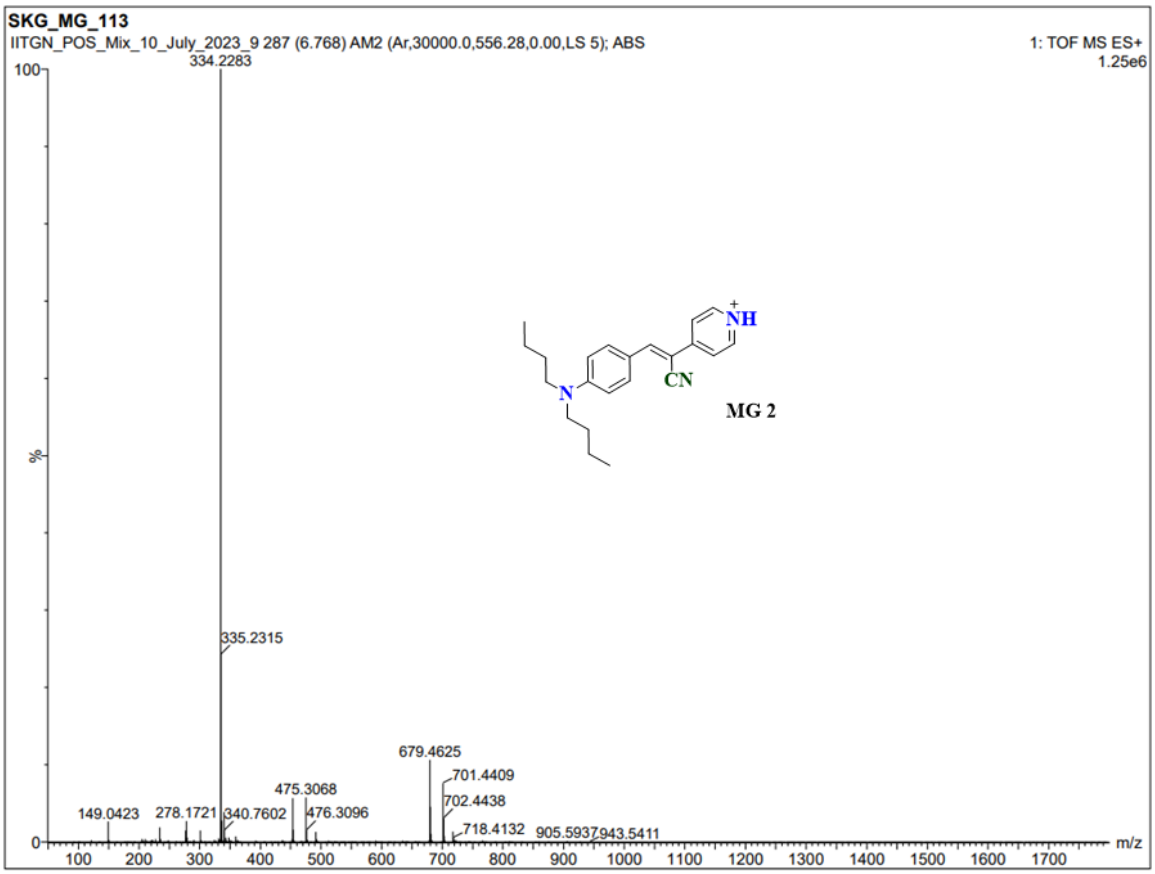
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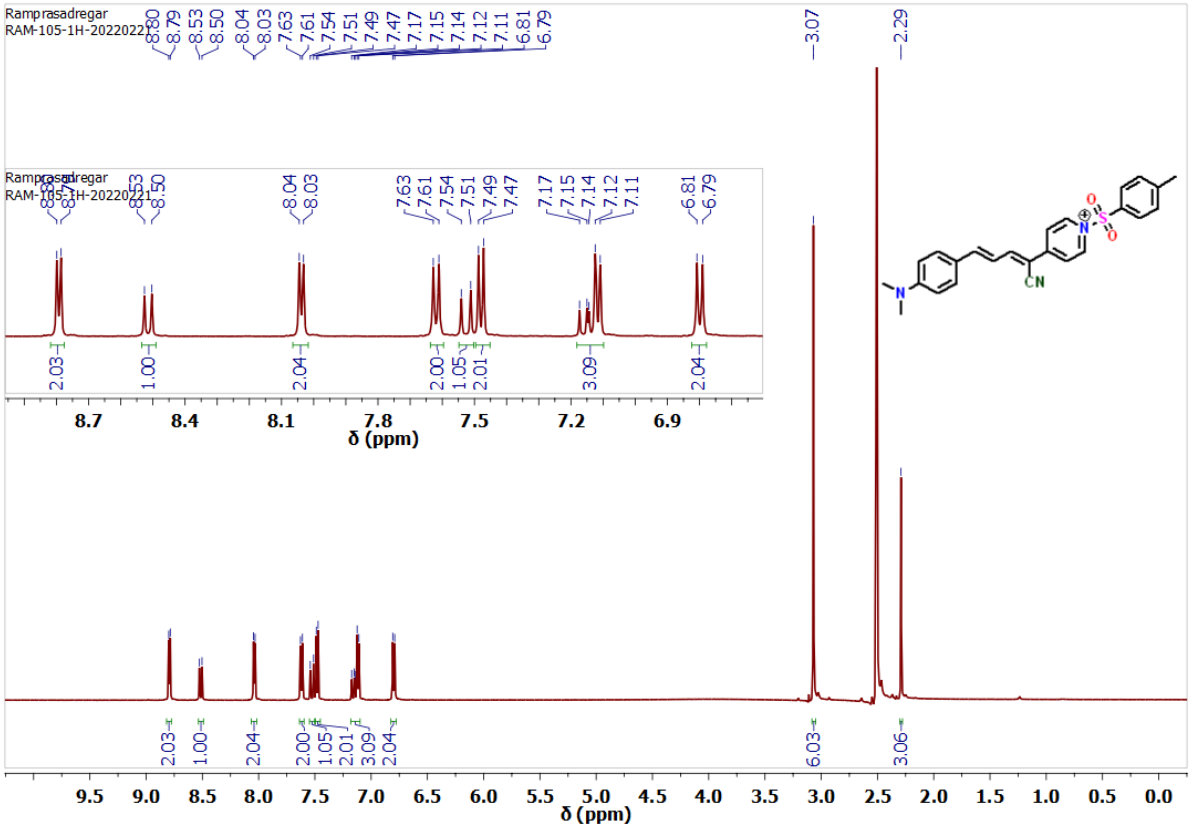
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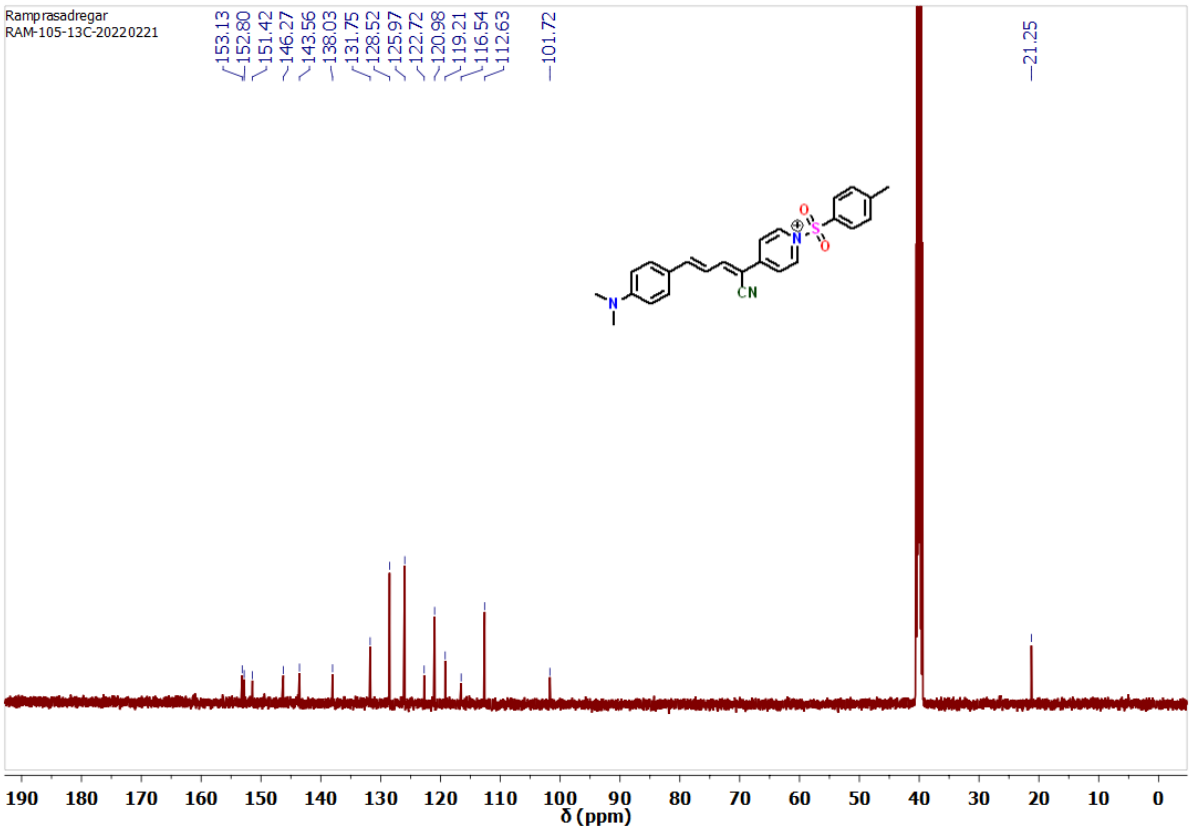
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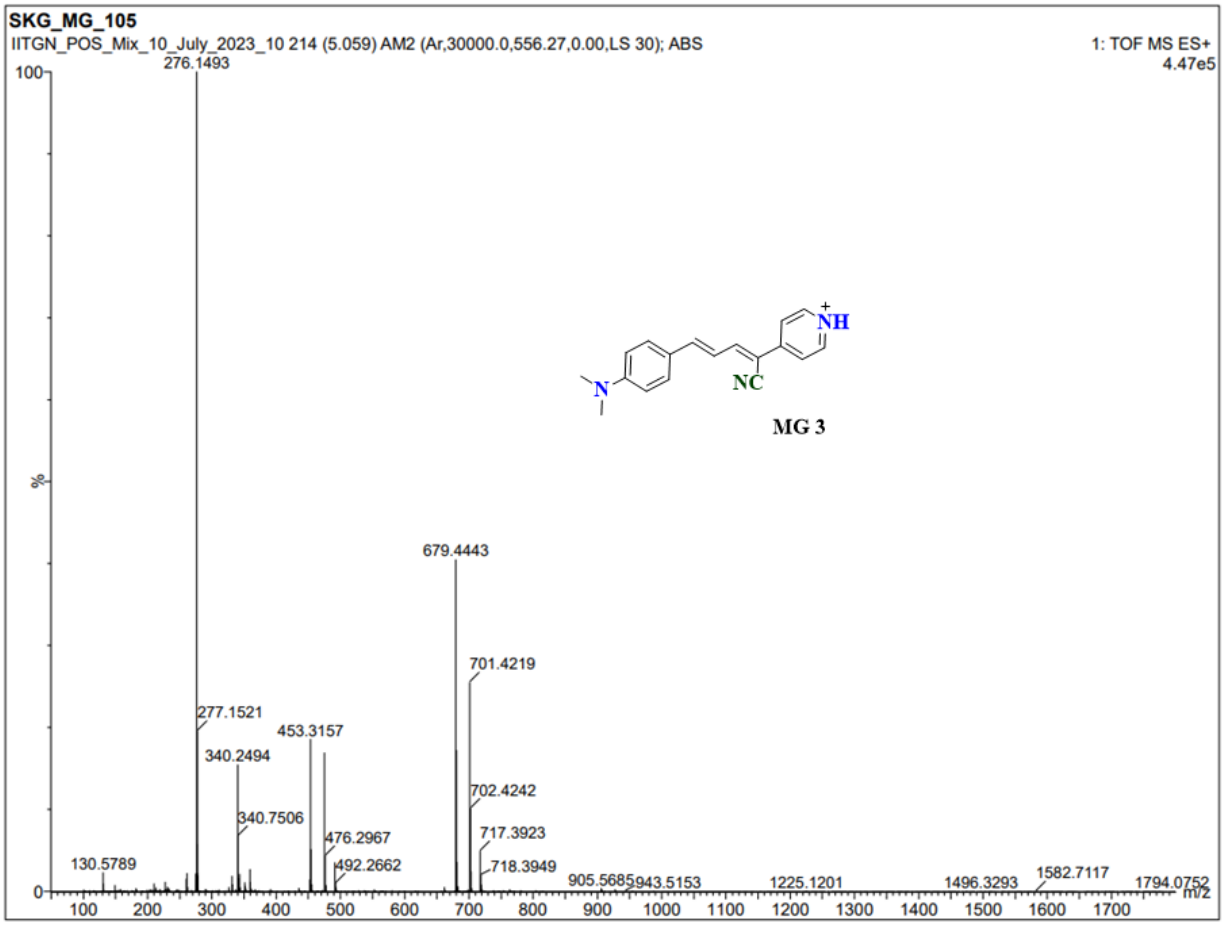
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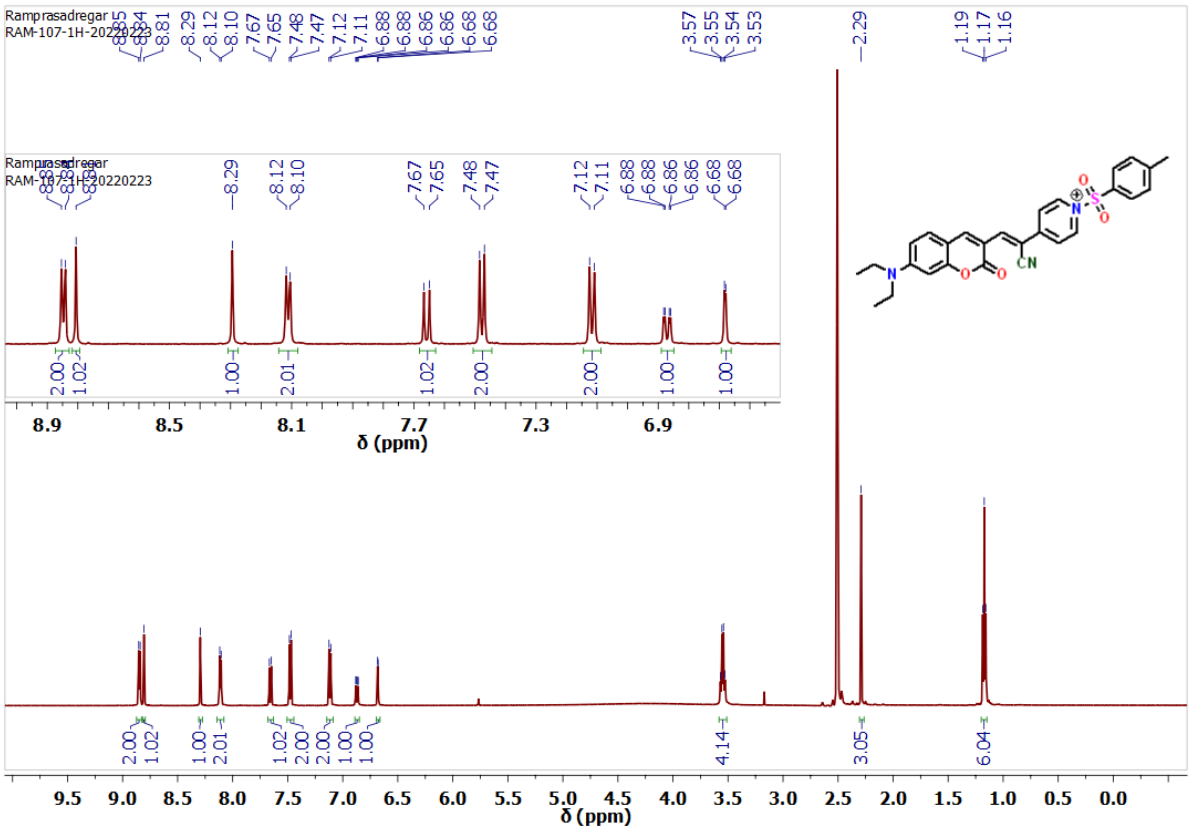
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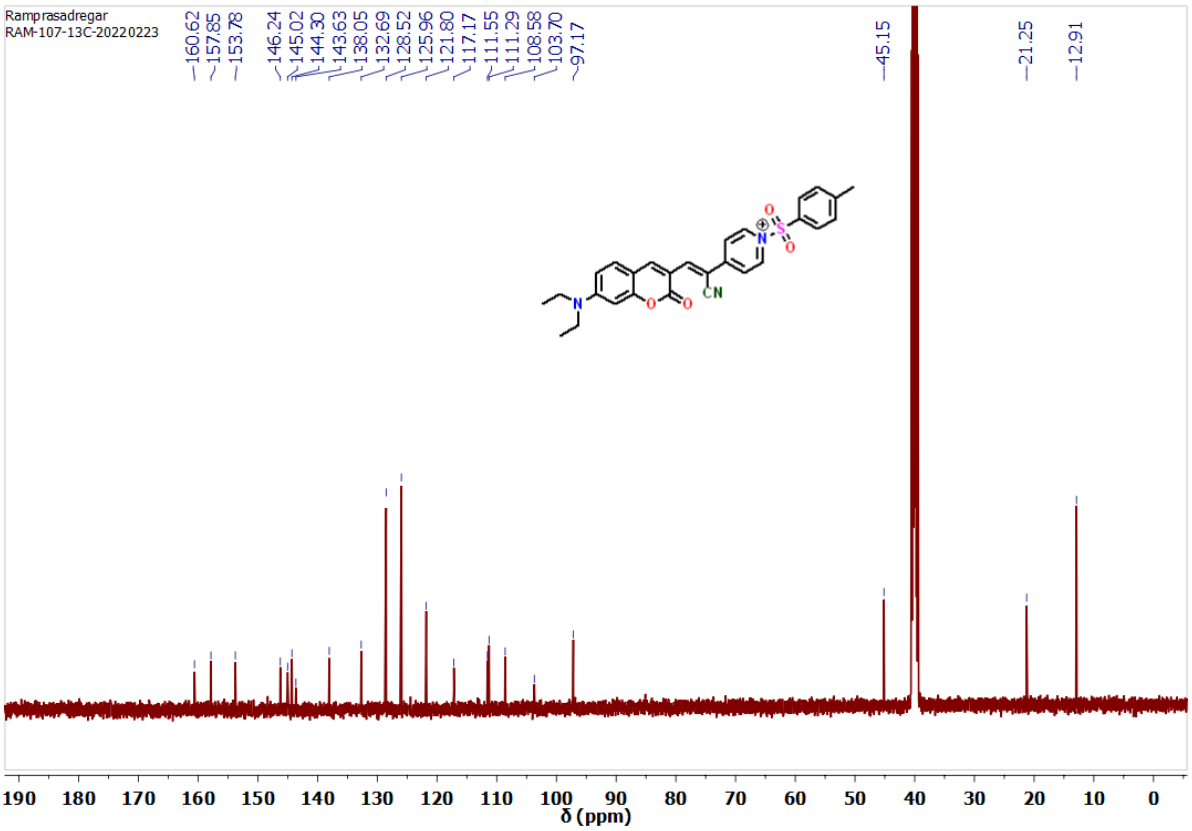
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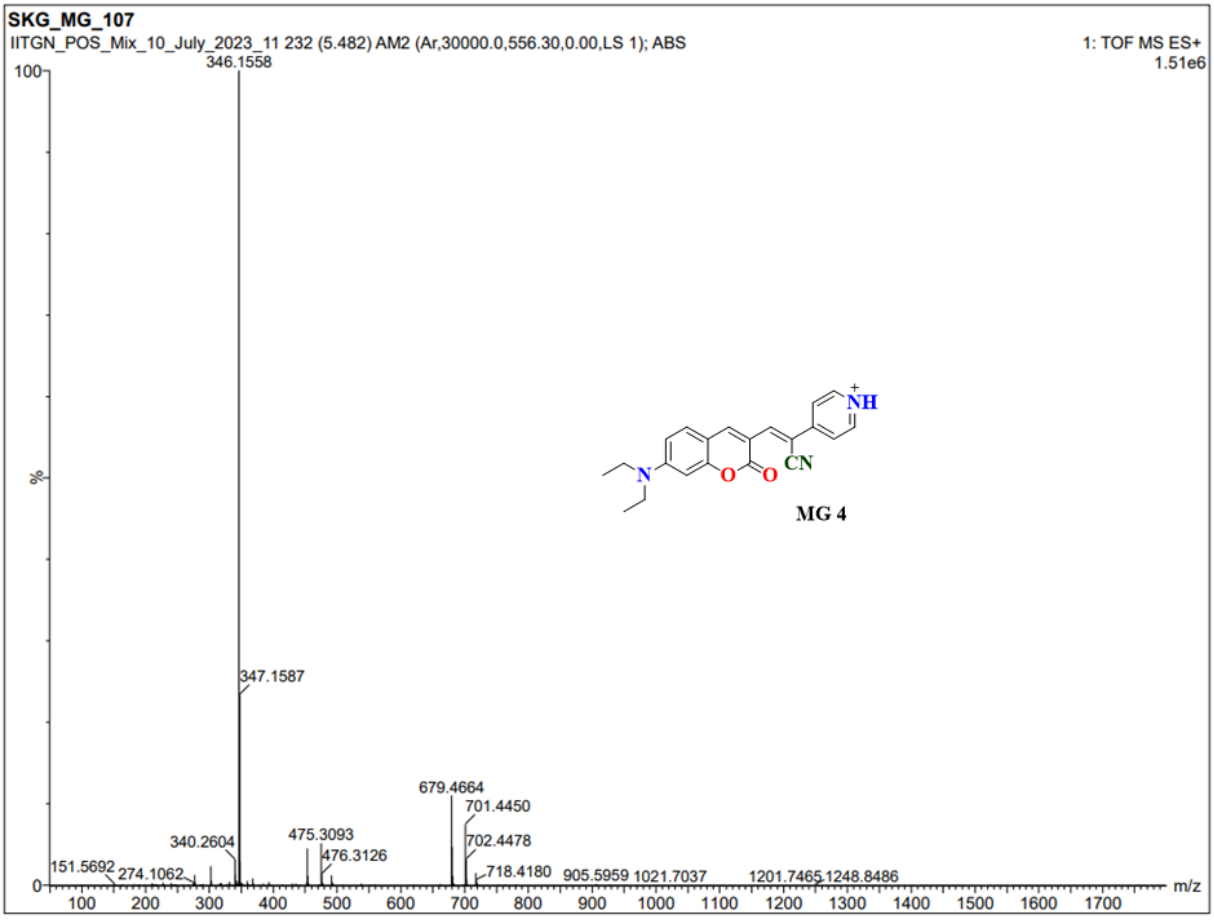
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