Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

From aggregation-induced to solution emission: A new strategy for designing ratiometric fluorescent probes and its application for *in vivo* HClO detection

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1. Fluorescence and UV-vis spectra affected by AIE

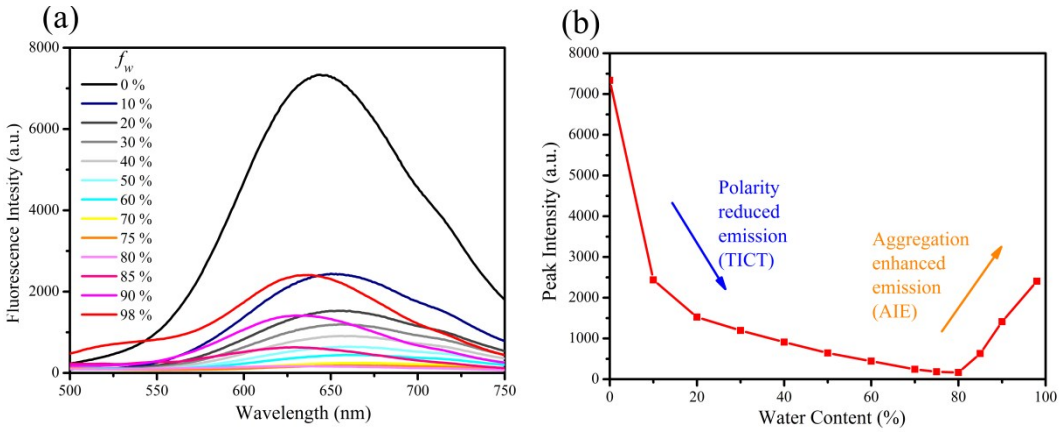


Fig. S1 (a) Fluorescence spectra of PDAM-Me in the MeCN-water mixtures with different water contents (λ_{ex} = 410 nm). (b) Fluorescence peak intensity of PDAM-Me *versus* water content of the solvent mixture.

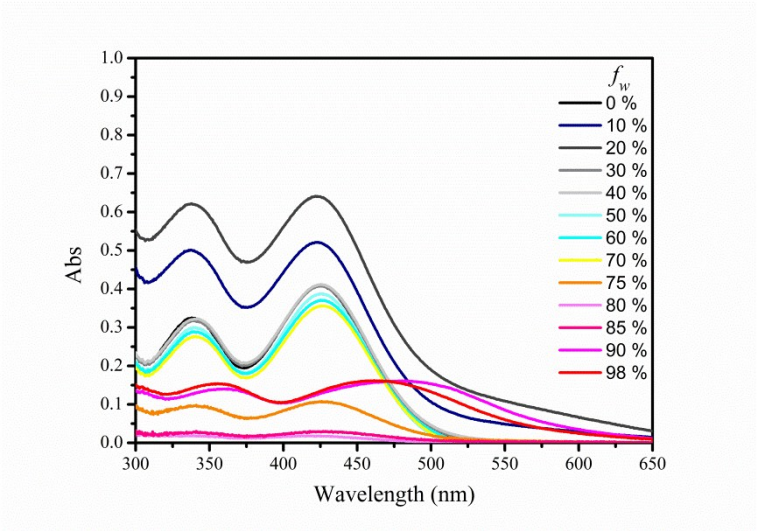


Fig. S2 UV-vis spectra of PDAM-Lyso (20 μM) in CH_3CN, upon increasing volume percentages of water from 0% to 98%.

2. Mechanism research

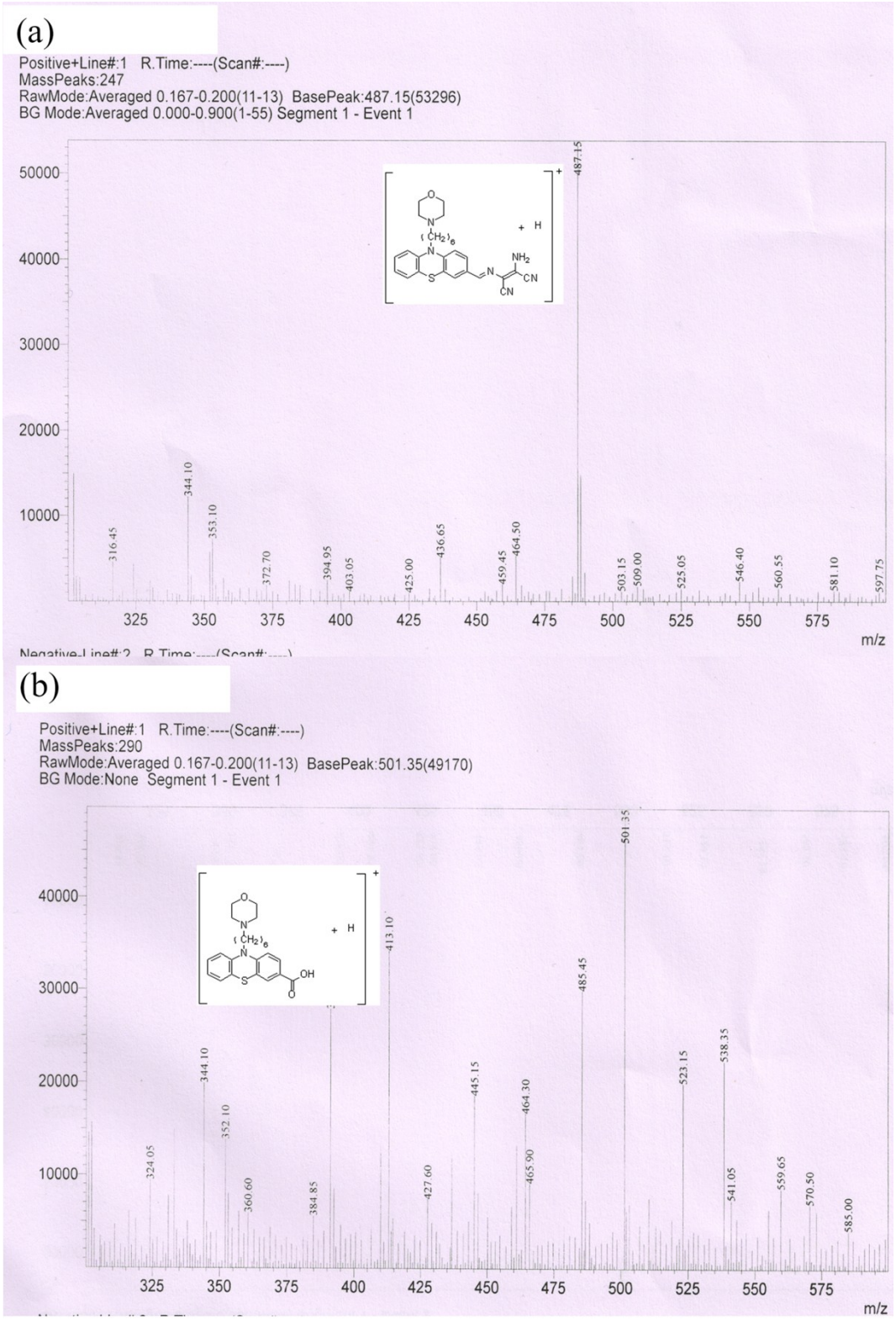


Fig.S3 (a) LCMS spectra of PDAM-Lyso. (b) LCMS spectra of PDAM-Lyso + NaClO.

3. Detection limit calculation

Fluorescence titration was carried out in CH_3CN/PBS buffer (1/9, v/v, 10 mM, pH = 7.4) to determine the detection limit, which was then calculated with the equation:

Detection of limit = 3bi/m

where bi is the standard deviation of blank measurements and is calculated with twenty (n = 20) experiments, and m is the slope between intensity ration (I_{470}/I_{620}) and sample concentration. It was calculated that the detection limit was 440 nm.

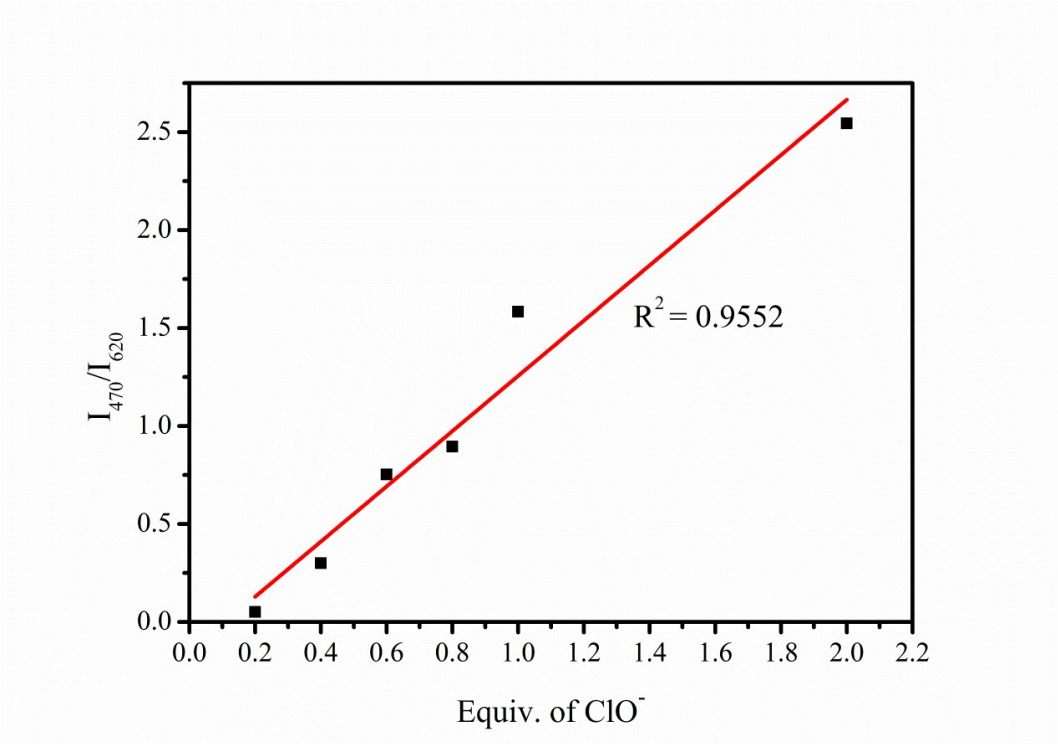


Fig. S4 The dependence curve of fluorescence intensity ratios (I_{470}/I_{620}) in the presence of ClO^- in CH_3CN/PBS buffer (1/9, v/v, 10 mM, pH = 7.4), (λ_{ex} = 410 nm).

4. Determination of reaction time

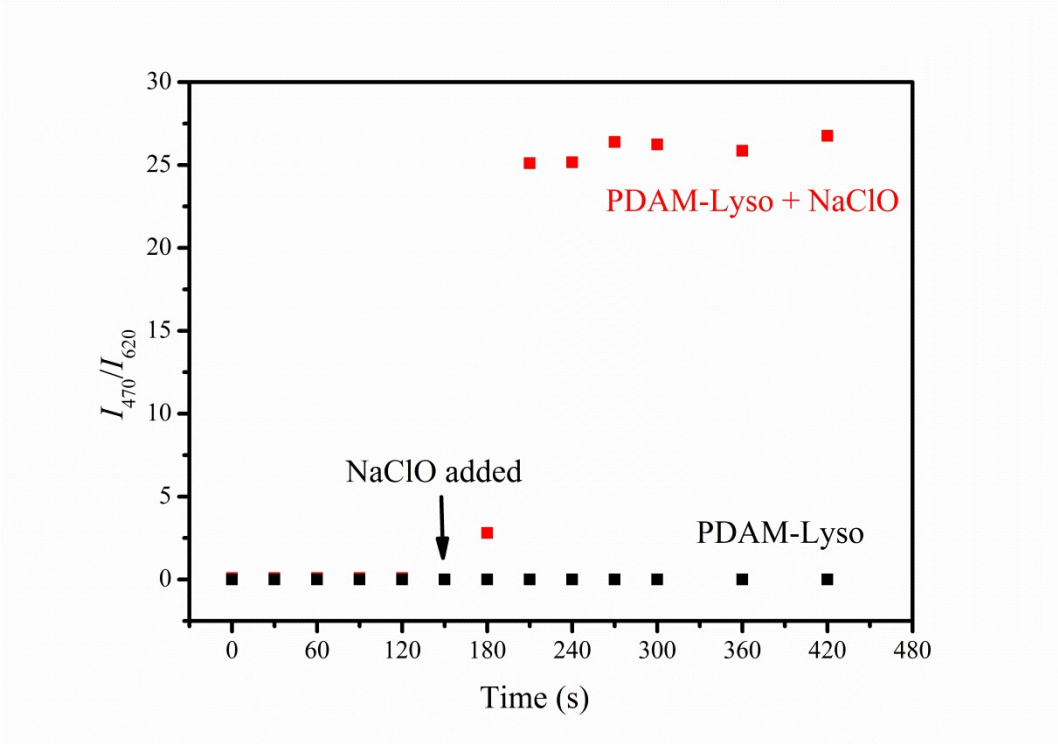


Fig. S5 Reaction-time profiles of PDAM-Lyso (20 μM) in the absence and presence of NaClO in CH_3CN/PBS buffer (1/9, v/v, 10 mM, pH = 7.4) (λ_{ex}=410 nm).

5. pH-dependent research

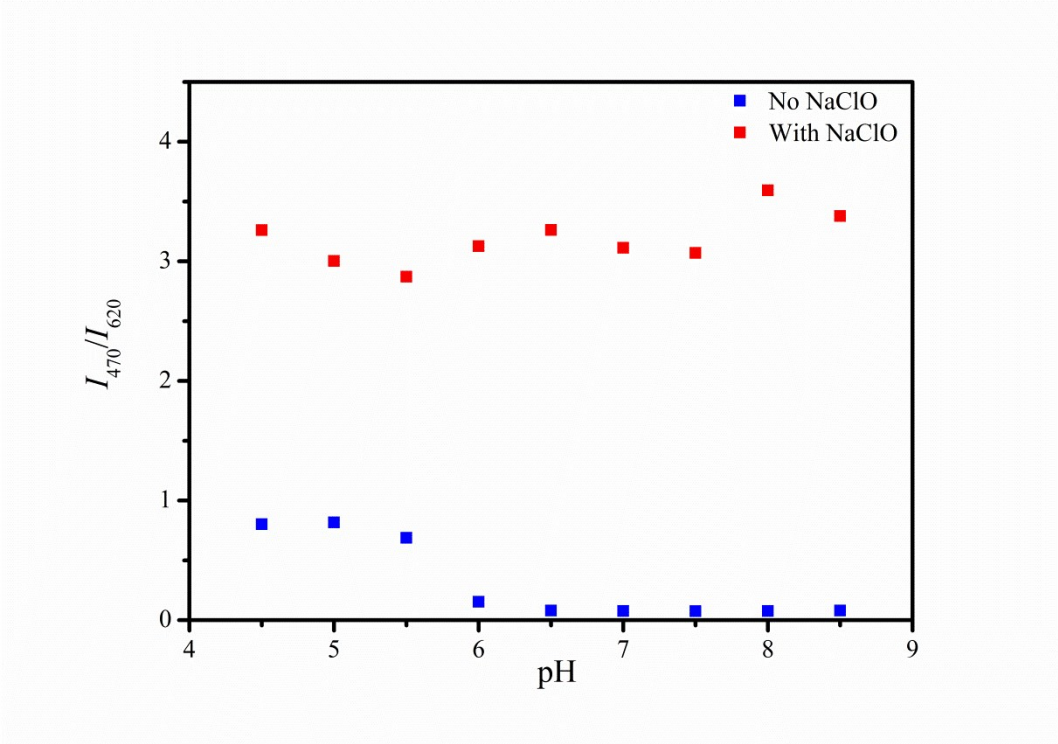


Fig. S6 pH-dependent profiles of PDAM-Lyso (20 μM) in the absence and presence of NaClO (2.5 eq.) with pH range of 4.5 – 8.5 in CH_3CN/PBS buffer (1/9, v/v, 10 mM) (λ_{ex}=410 nm).

6. Cytotoxicity study

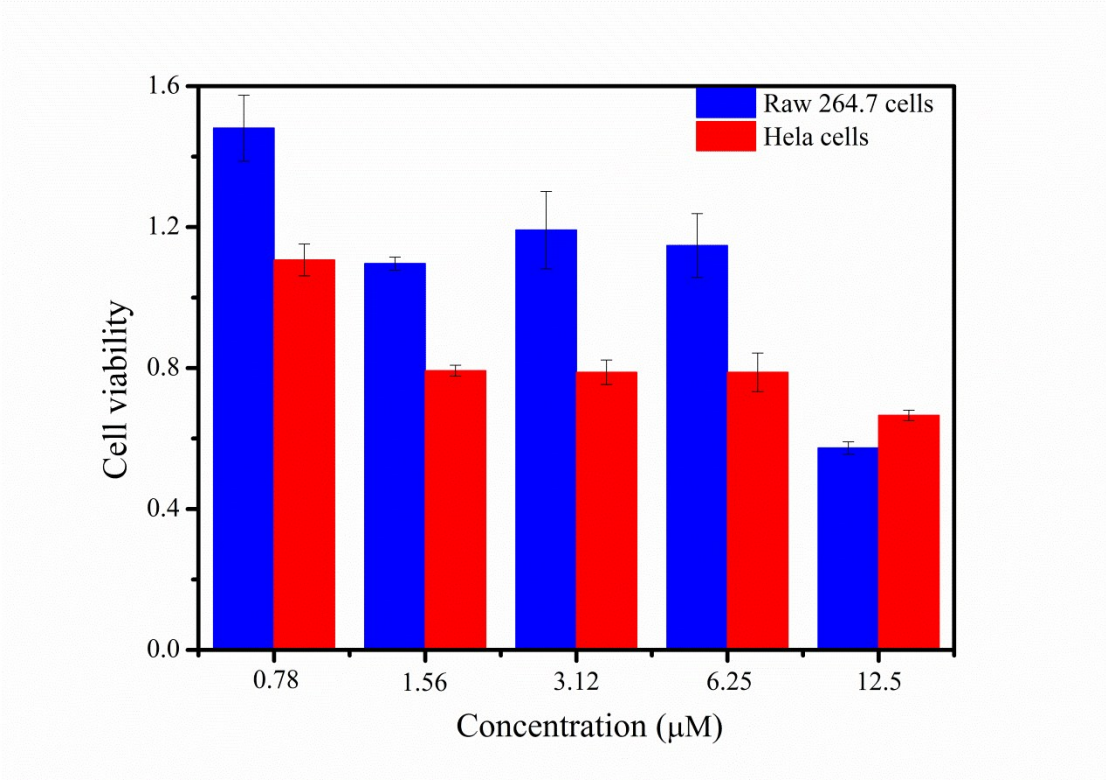


Fig. S7 Cytotoxicity data of PDAM-Lyso for Raw264.7 cells (blue) and Hela cells (red).

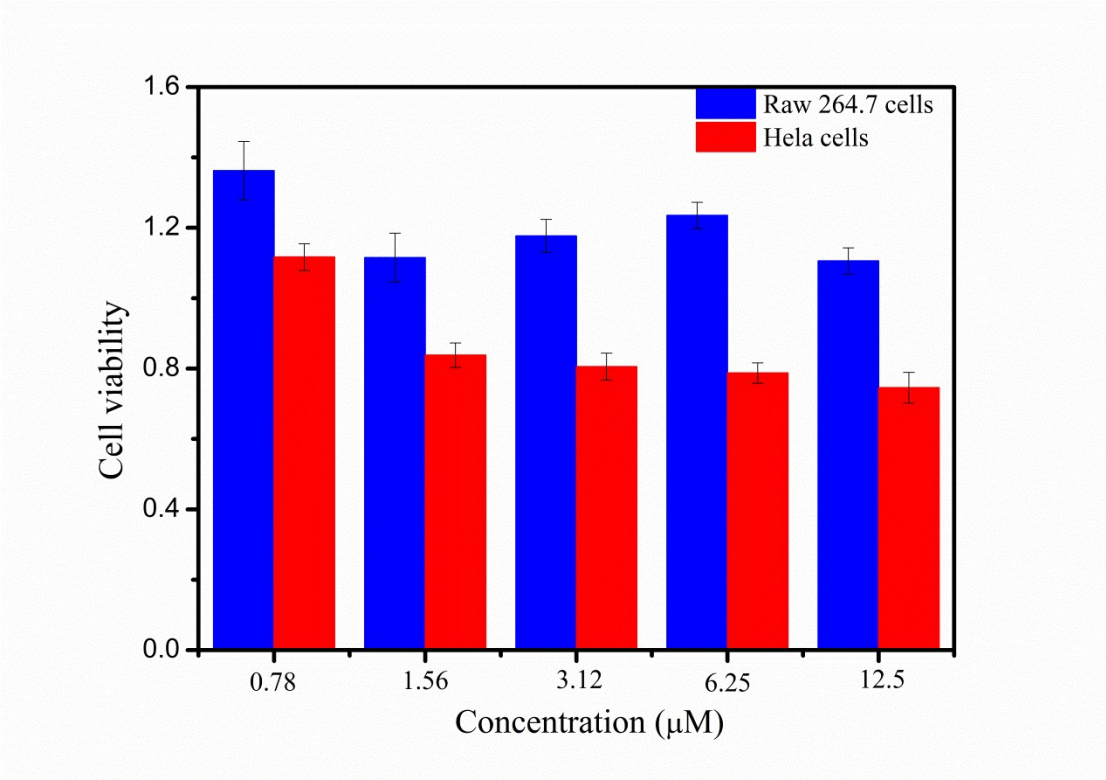


Fig. S8 Cytotoxicity data of PDAM-Me for Raw264.7 cells (blue) and Hela cells (red).

7. Cell imaging

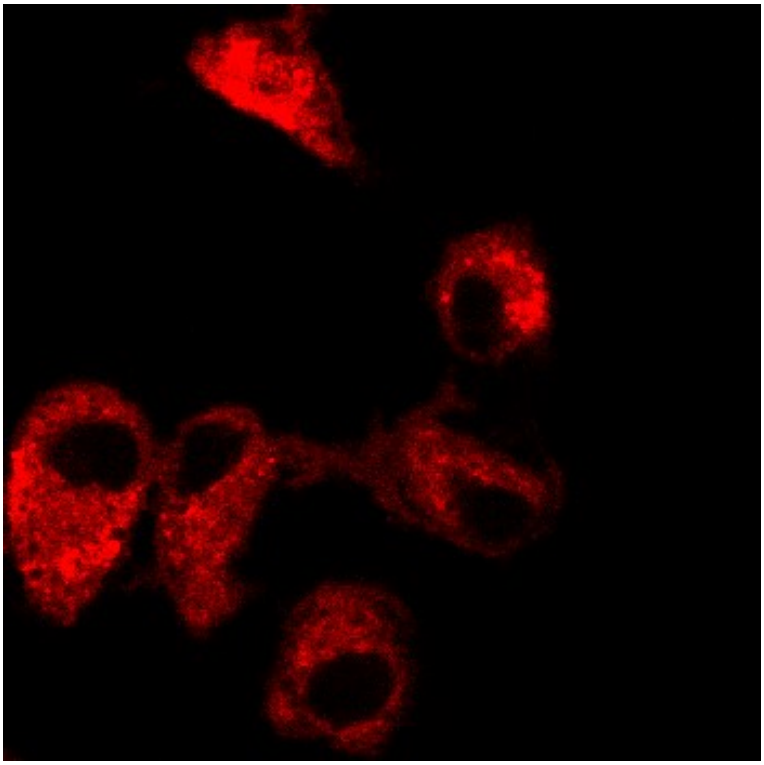


Fig. S9 Confocal fluorescent images of Hela cells of PDAM-Lyso and Lyso-Tracker red (λ_{ex} = 405 nm, λ_{em} = 560 – 690 nm).

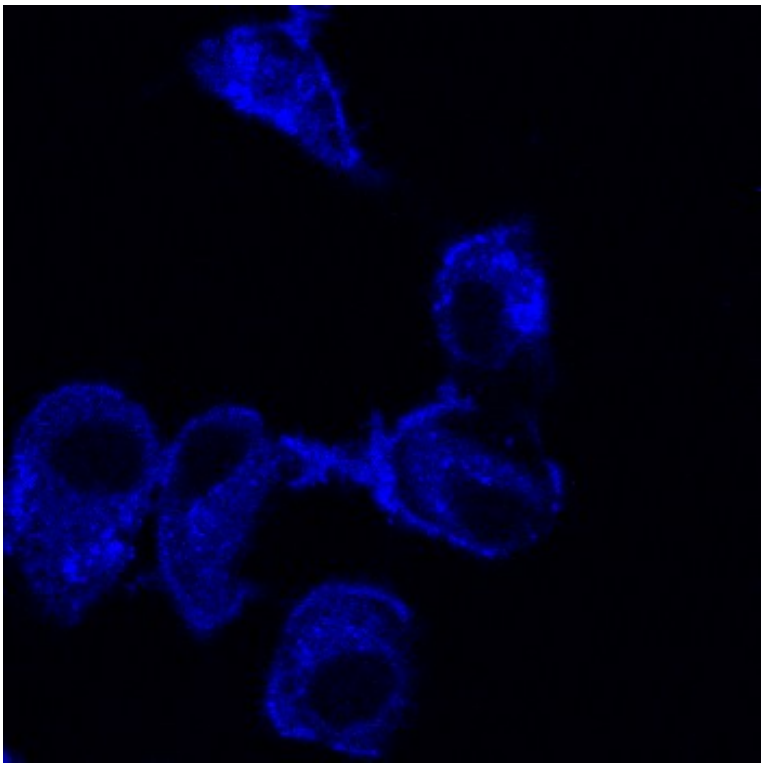


Fig. S10 Confocal fluorescent images of Hela cells of PDAM-Lyso and Lyso-Tracker red (λ_{ex} = 405 nm, λ_{em} = 430 – 560 nm).

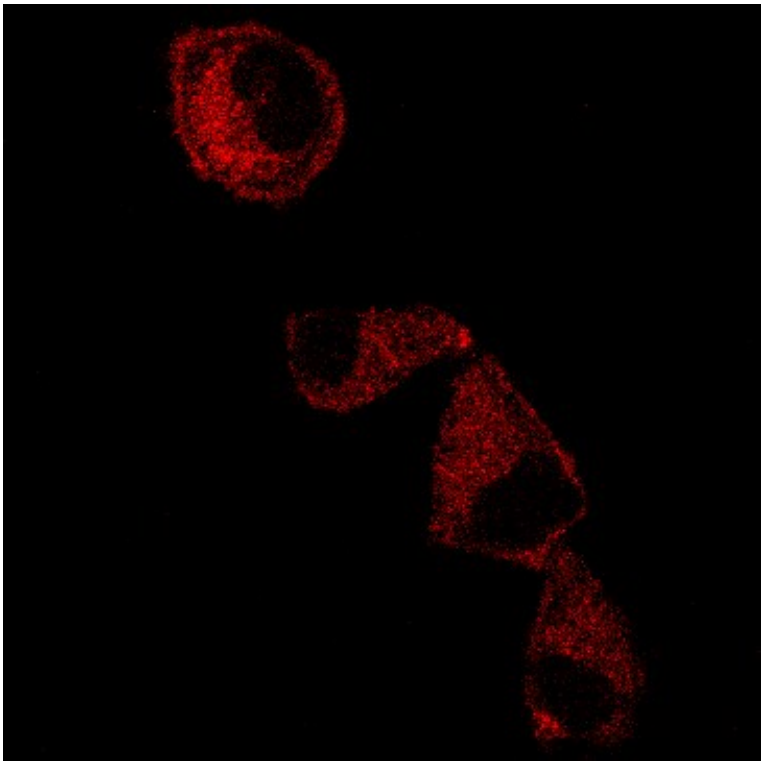


Fig. S11 Confocal fluorescent images of Hela cells of PDAM-Me and Lyso-Tracker red (λ_{ex} = 405 nm, λ_{em} = 560 – 690 nm).

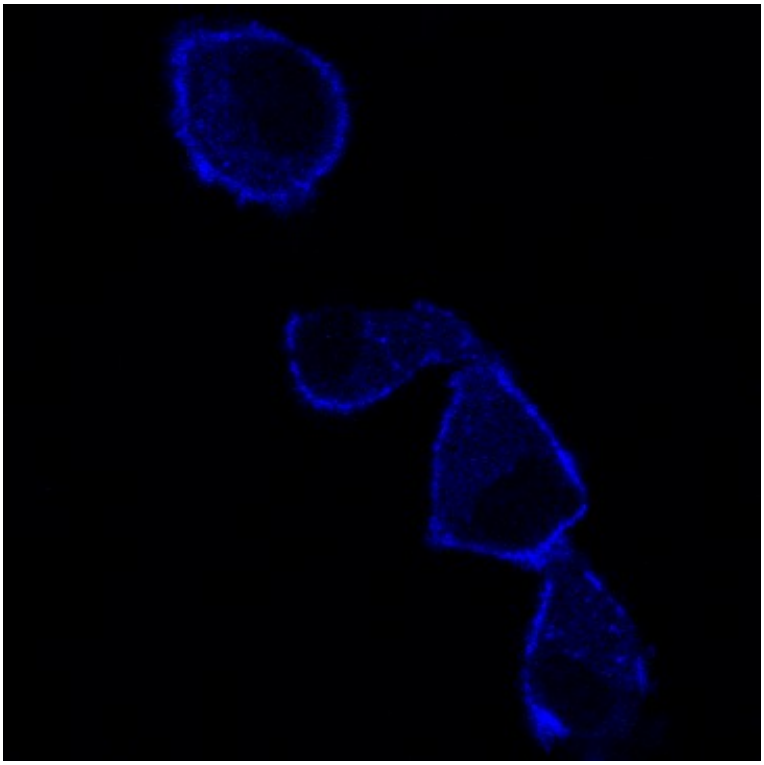


Fig. S12 Confocal fluorescent images of Hela cells of PDAM-Me and Lyso-Tracker red (λ_{ex}= 405 nm, λ_{em} = 430 – 560 nm).

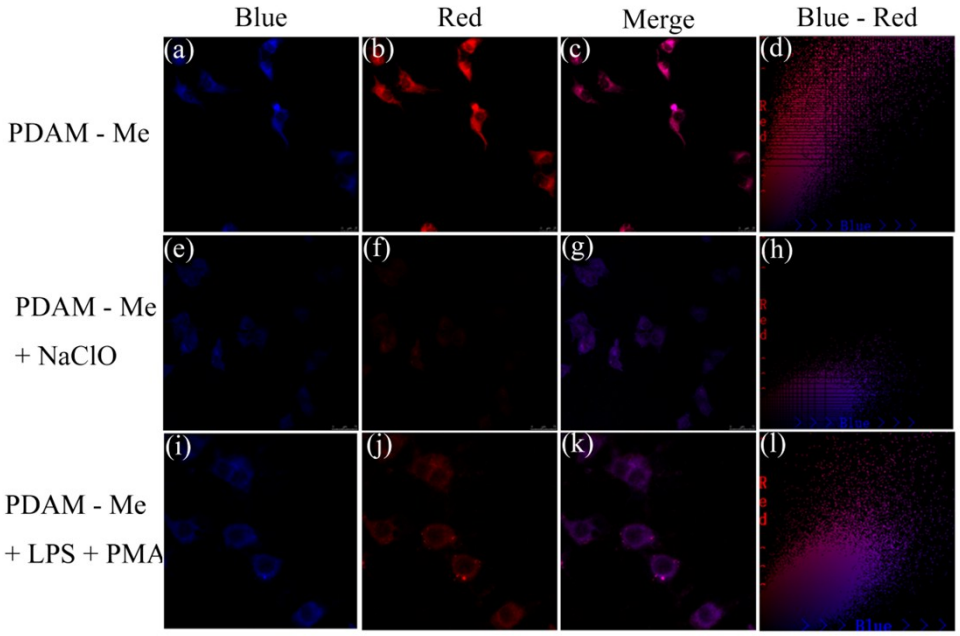


Fig. S13 Confocal fluorescence images of PDAM-Me in cells(λ_{ex} = 410 nm). (a) Hela cells, PDAM-Me (3.0 μM), 420 – 550 nm; (b) Hela cells, PDAM-Me (3.0 μM), 570 – 700 nm; (c) The overlay of (a) and (b); (d) The scatter distributing plot of (c). (e) Hela cells, PDAM-Me (3.0 μM), NaClO (60μM), 420 – 550 nm; (f) Hela cells, PDAM-Me (3.0 μM), NaClO (60 μM), 570 – 700 nm; (g) The overlay of (e) and (f); (h) The scatter distributing plot of (g). (i) Raw 264.7 cells, PDAM-Me (3.0 μM), LPS (1.0 μg/mL), PMA (1.0 μg/mL), 420 – 550 nm; (j) Raw 264.7 cells, PDAM-Me (3.0 μM), LPS (1.0 μg/mL), PMA (1.0 μg/mL), 570 – 700 nm; (k) The overlay of (i) and (j); (l) The scatter distributing plot of (k).

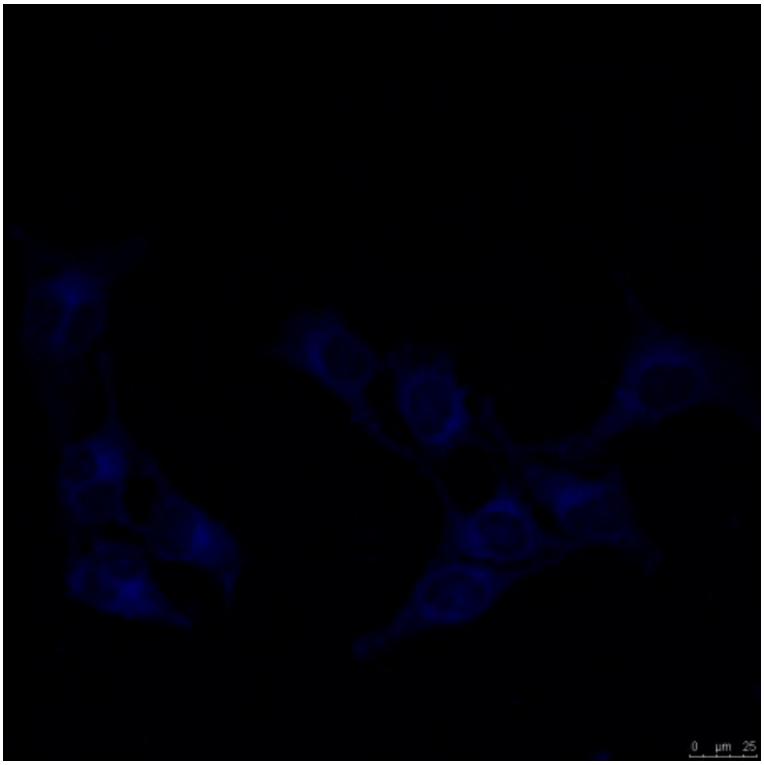


Fig. S14 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells (λ_{ex} = 410 nm, λ_{em} = 420 – 550 nm).

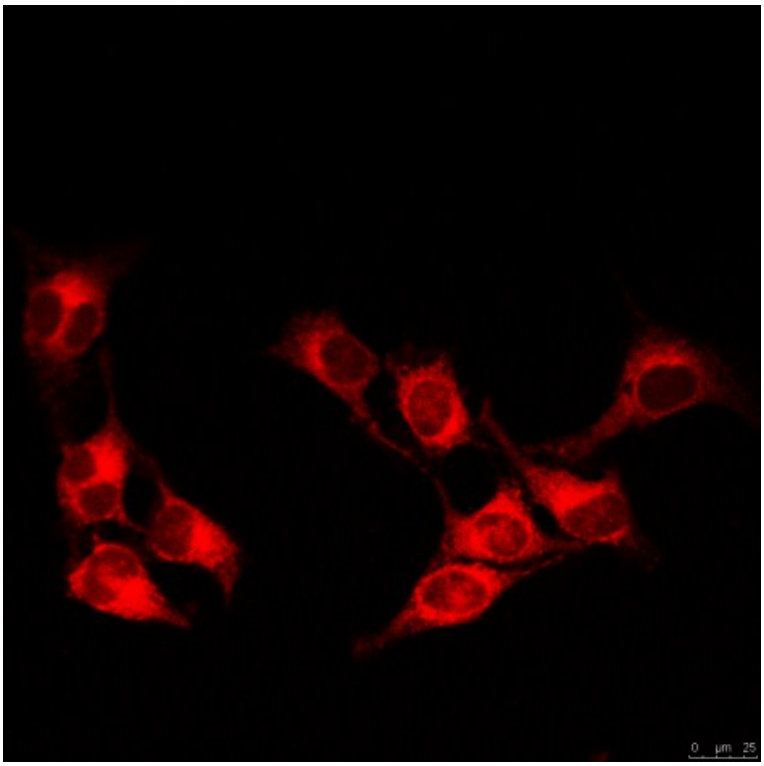


Fig. S15 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells (λ_{ex} = 410 nm, λ_{em} = 570 – 700 nm).

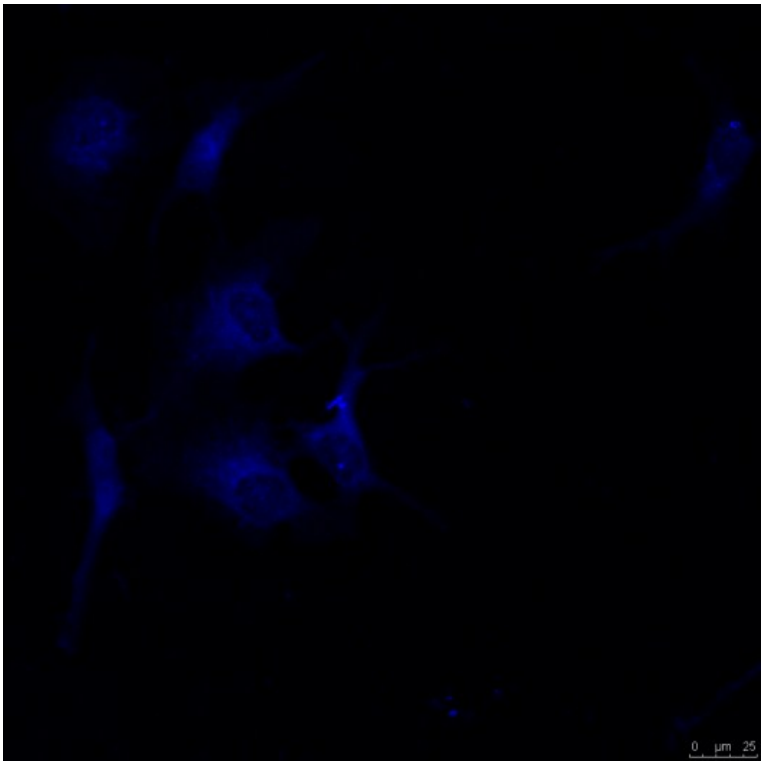


Fig. S16 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells with the existence of NaClO (60 μM) (λ_{ex} = 410 nm, λ_{em} = 420 – 550 nm).

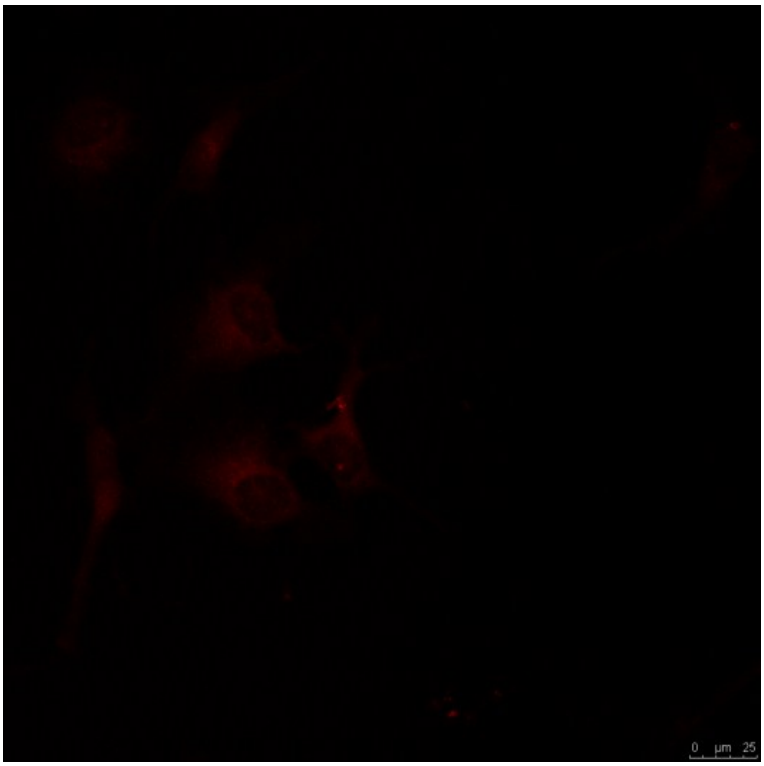


Fig. S17 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Hela cells with the existence of NaClO (60 μM) (λ_{ex} = 410 nm, λ_{em} = 570 – 700 nm).

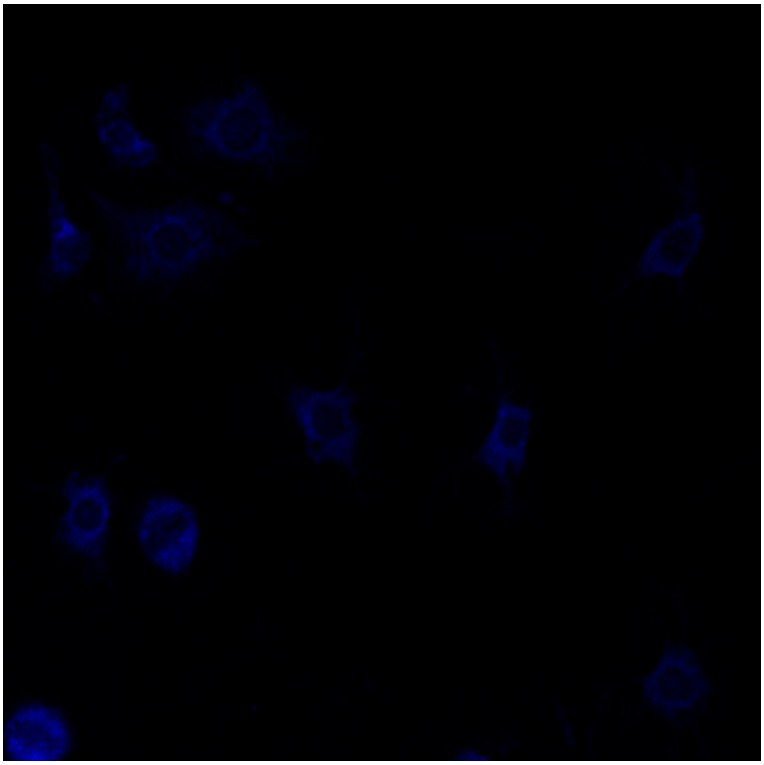


Fig. S18 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Raw 264.7 cells with LPS (1.0 μg/mL) and PMA (1.0 μg/mL) cells (λ_{ex} = 410 nm, λ_{em} = 420 – 550 nm).

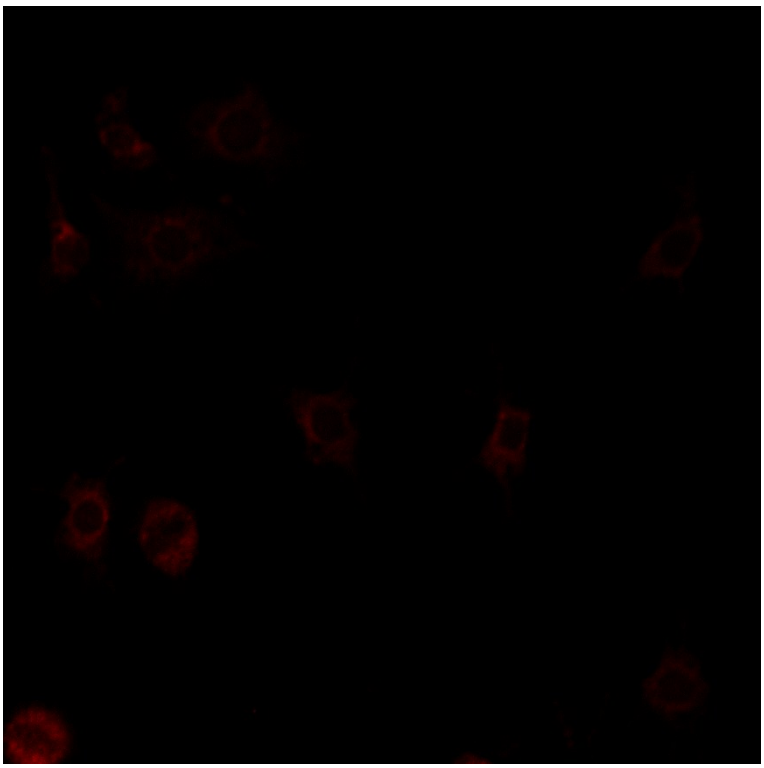


Fig. S19 Confocal fluorescent images of PDAM-Lyso (3.0 μM) in Raw 264.7 cells with LPS (1.0 μg/mL) and PMA (1.0 μg/mL) cells (λ_{ex} = 410 nm, λ_{em} = 570 – 700 nm).

8. Crystal data of PDAM-Me

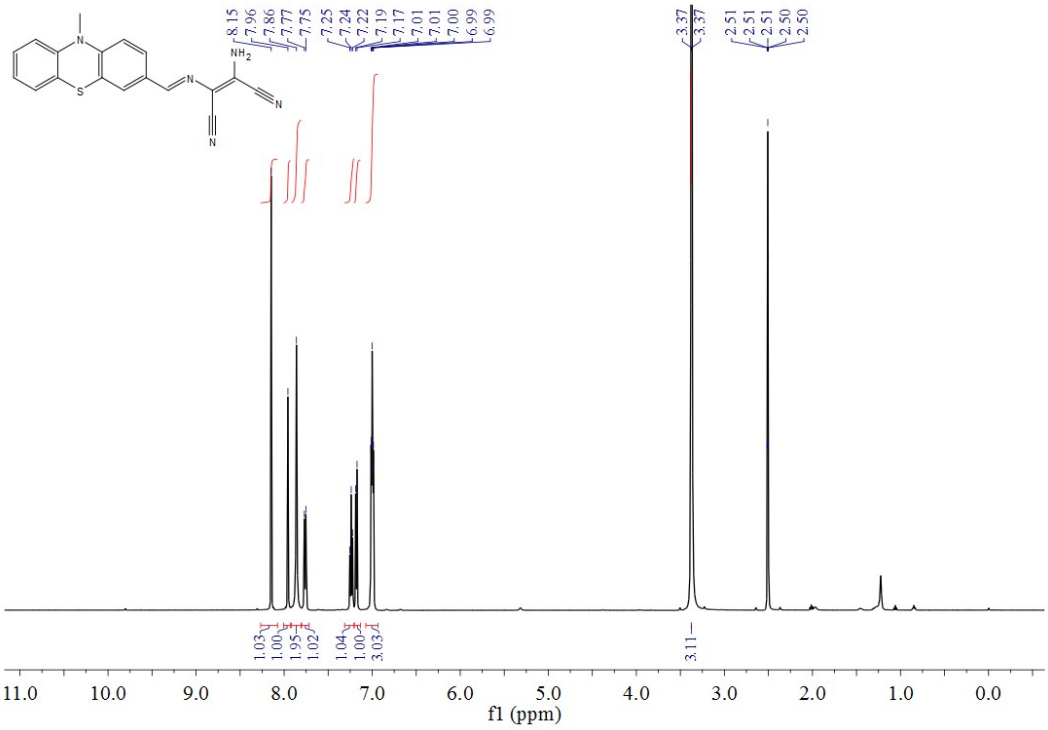
Table S1 Crystal data of compound PDAM-Me

Compound	PDAM-Me
Empirical formula	$C_{18}H_{13}N_5S$
Formula weight	331.39
Crystal system	monoclinic
Space group	$P2_1/c$
a[Å]	18.09(2)
b[Å]	6.977(9)
c[Å]	13.863(19)
α[°]	90
β[°]	109.407(12)
γ[°]	90
V[Å^3]	1650(4)
Z	4
T[K]	291(2)
D_{calcd}[g·cm^{-1}]	1.334
F(000)	688
μ[mm^{-1}]	0.205
θ range[°]	2.388 - 24.999
R_1	0.1562
wR_2	0.4408
S	1.124

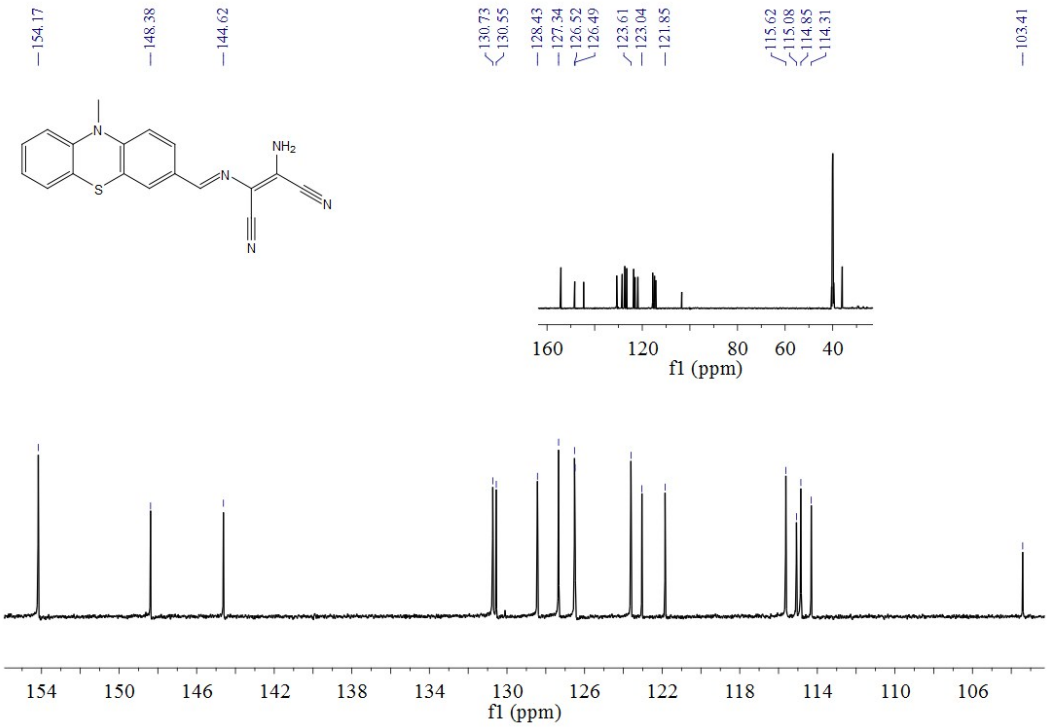
Table S2 Intermolecular hydrogen bonding parameters (Å, °) in PDAM-Me

D–H···A	D–H	H···A	D···A (d)	∠DHA	Symmetry code
N5–H5A···N3	0.86	2.22	3.031(15)	157	x, -1+y, z
N5–H5B···N4	0.86	2.19	3.028(14)	166	-x, -1/2+y, 1/2-z

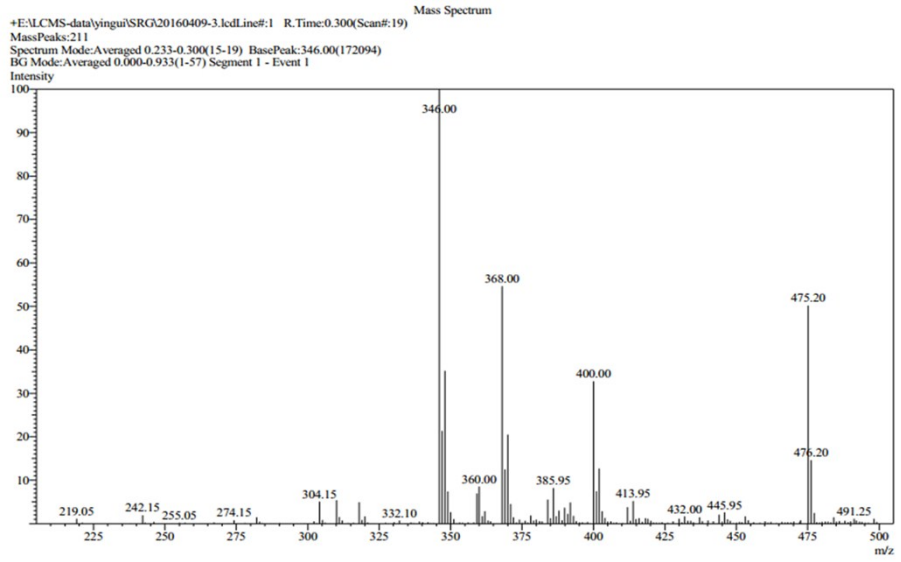
9. Supplemental spectra



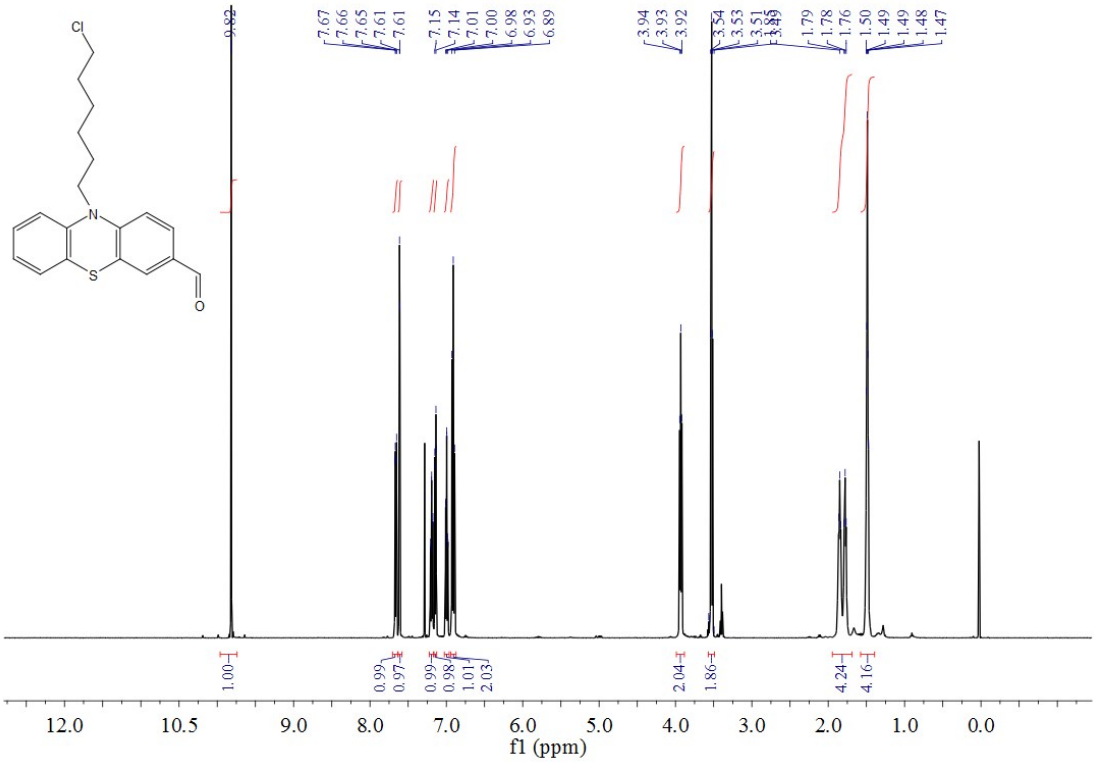
^{1}H NMR spectrum of PDAM-Me.



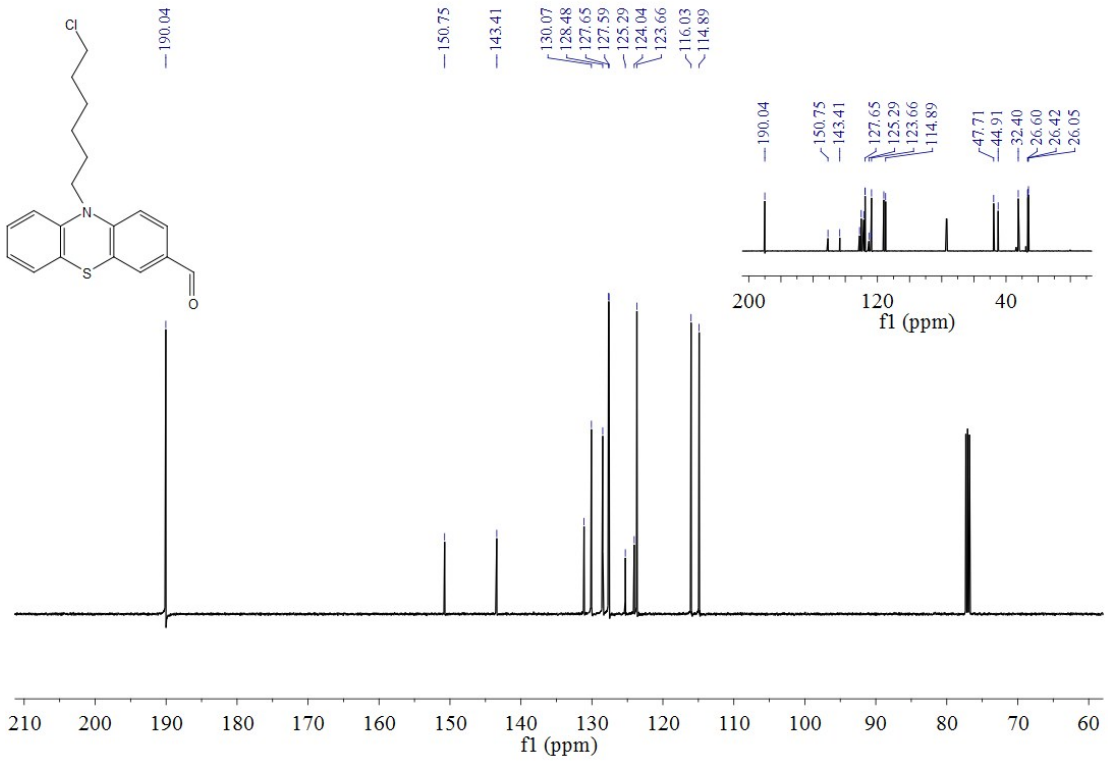
^{13}C NMR spectrum of PDAM-Me.



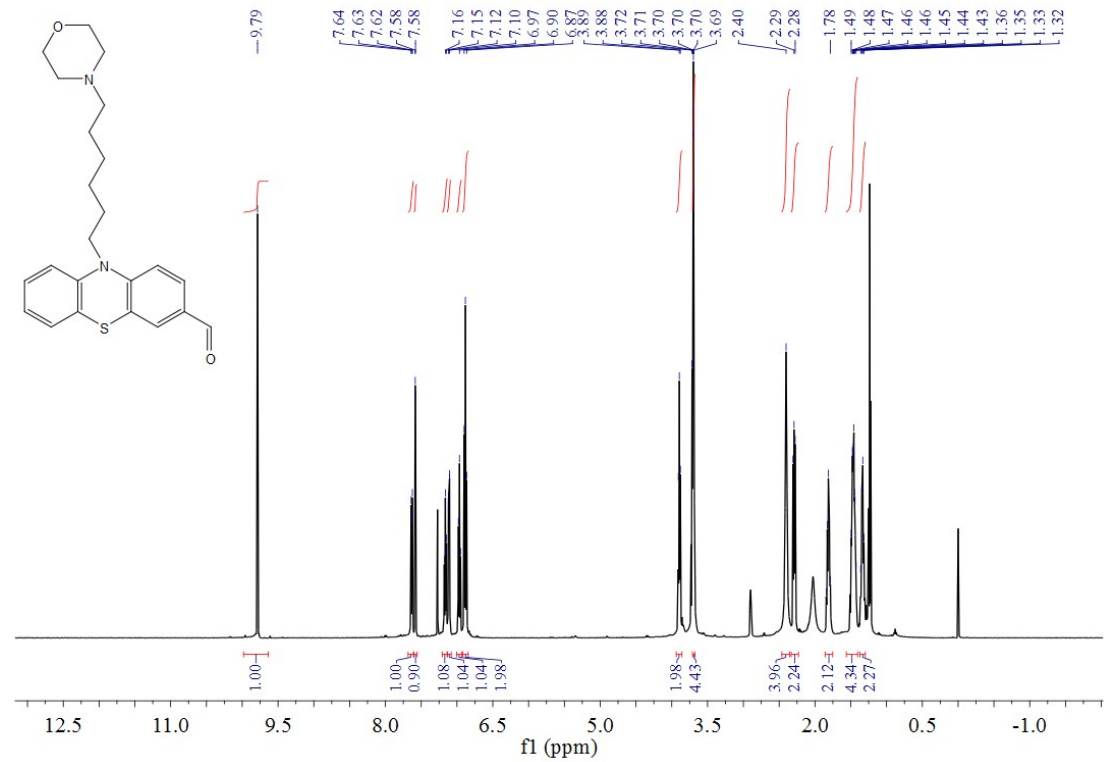
ESI-MS spectrum of the compound 4.



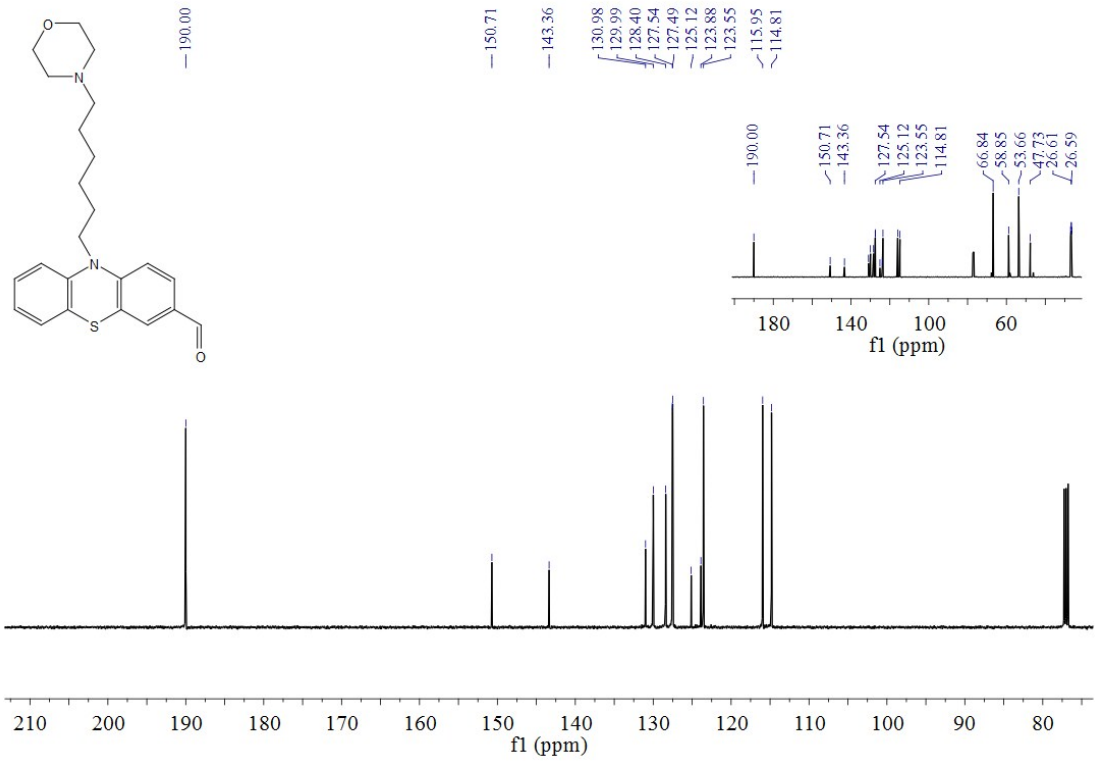
^{1}H NMR spectrum of compound 4.



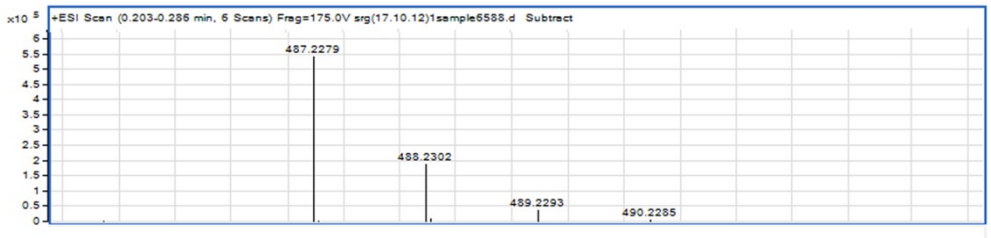
^{13}C NMR spectrum of compound 4.



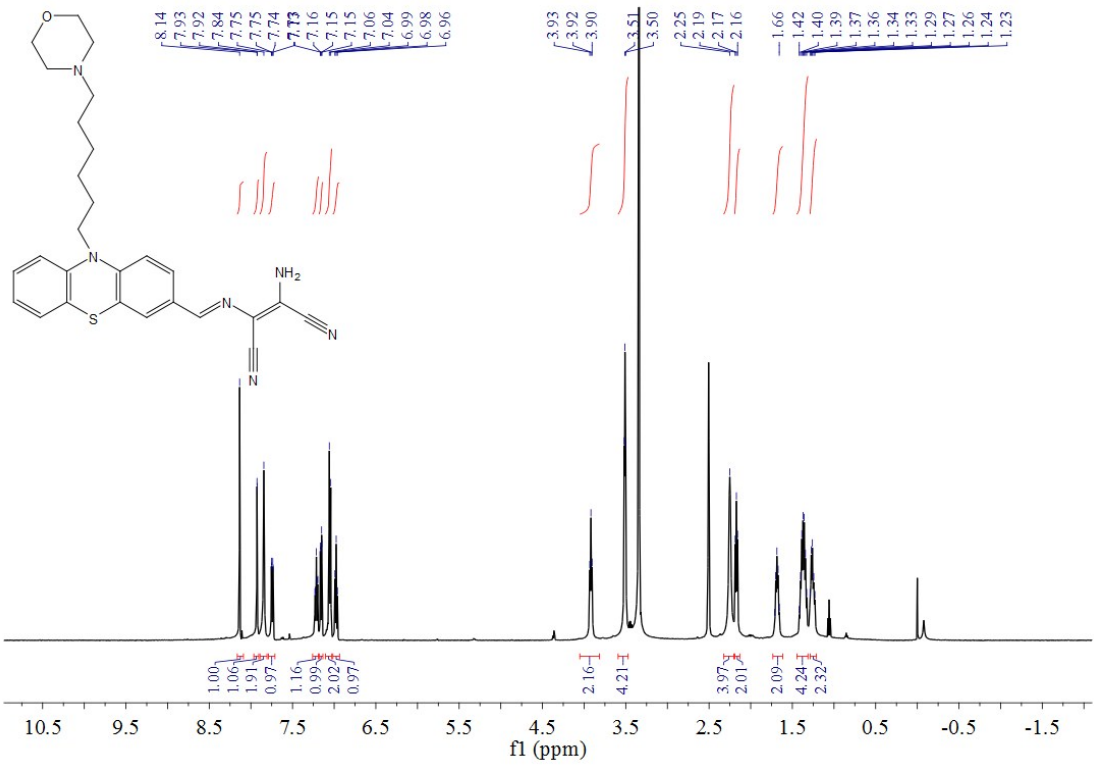
^{1}H NMR spectrum of compound 5.



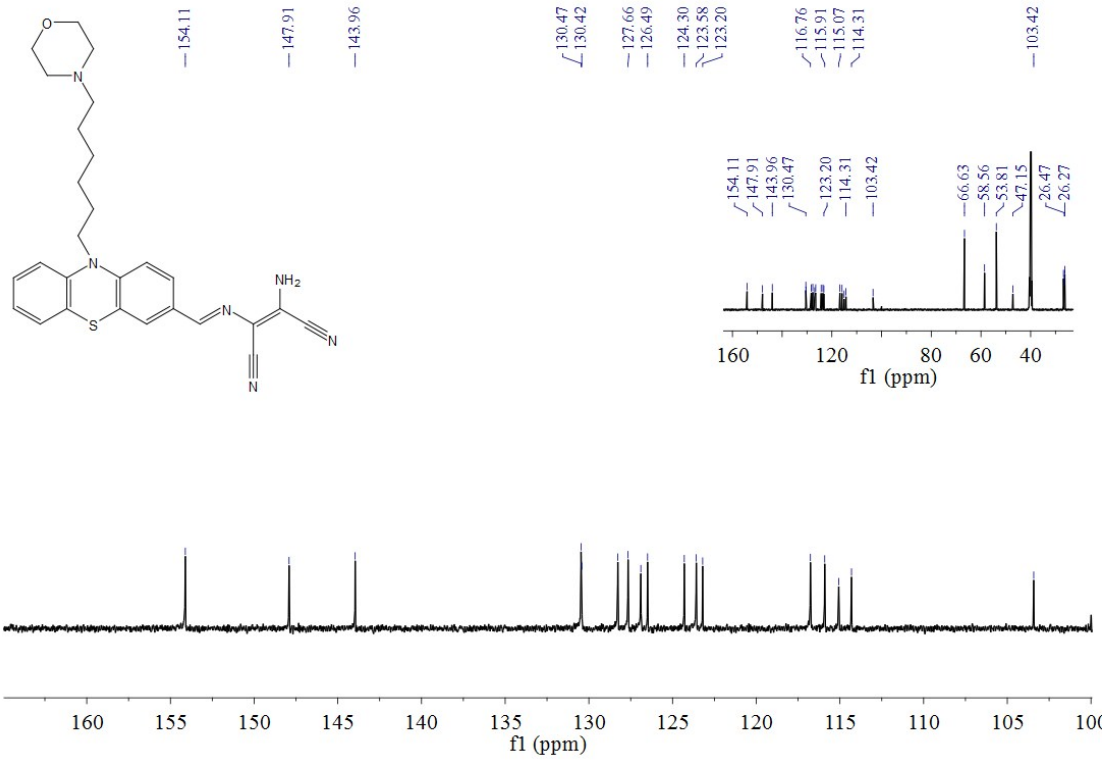
^{13}C NMR spectrum of compound 5.



HR-MS spectrum (positive) of PDMA-Lyso.



^{1}H NMR spectrum of PDMA-Lyso.



^{13}C NMR spectrum of PDMA-Lyso.