An efficient PeT based fluorescent probe for mapping mitochondrial oxidative stress produced via the Nox2 pathway

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Table of Contents

1. General information

α-Tetralone and Benzaldehyde were obtained from Sigma Aldrich (MO, USA). 4-methylthio benzaldehyde and Methylamine, 40% in methanol were purchased from TCI, Tokyo, Japan. Phosphate buffer saline (PBS) tablets were procured from Loba Chemicals, Mumbai, India. Ammonia solution was purchased from Rankem, India. Perchloric acid 60% was obtained from Thermo Fischer, India. Dimethyl sulphoxide (DMSO) and other HPLC grade solvents were obtained from Chemlabs, Mumbai, India. DMSO-*d6* was procured from Eurisotop, France. Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS), and 100 U penicillin /0.1 mg/ml streptomycin antibiotics were also procured from HiMedia Laboratories, Mumbai and Gibco, Thermo Fisher, UK respectively. Reaction completion was monitored by silica gel G-60 F254 aluminium TLC and compounds were visualized by short/long-wavelength UV lamps. Column chromatography was done using silica gel 100-200 mesh. Distilled MilliQ water was used for all experiments. Unless otherwise mentioned all the chemicals and the solvents were used as obtained without further purifications.

2. General Instrumentations

The NMR spectra of the synthesized molecules were studied in the Bruker Avance 400 NMR spectrometer, Germany, after diluting in deuterated solvents. The mass spectrometric analysis was studied in Agilent 6540, Q-TOF LC/MS system (Agilent Technologies, Santa Clara, CA, USA) connected with Agilent 1290 UPLC. DI water and acetonitrile along with 0.1% TFA were used as mobile phase with gradient solvent system. The absorption spectra were measured in an Agilent Cary 8454 UV-Vis diode array spectrophotometer. The photoluminescence spectra were measured in Edinburgh FS5 spectrofluorometer (Edinburgh Instruments, Livingstone, United Kingdom; 150 W CW ozone free xenon arc lamp with fully automated filter wheels in the excitation and emission monochromators) and with a HORIBA Fluorolog-3 spectrofluorometer (Model: FL3-2-IHR). The scan slits for excitation and emission were adjusted to 1 nm. For biological experiments, cells were incubated in a Thermo Fisher CO_2 incubator and the images were captured in a NIKON Ti-U inverted fluorescence microscope and with a Nikon confocal microscope.

3. Synthesis of PY-P

An oven-dried round bottom flask was charged with benzaldehyde (3.42 mmol) and α-Tetralone (6.84 mmol) in 10 ml Toluene and stirred at 110 ^{o}C for 30 minutes. Then perchloric acid (3.42 mmol) was added dropwise and the solution was stirred for one and a half hours.

Black sticky precipitation was visible at the bottom of the flask. After completion of the reaction, the mixture was cooled down to room temperature and the toluene part was decanted so that only the black precipitation remains in the round bottom flask. This precipitation was dissolved in a minimum amount of methanol and diethyl ether (10 mL) was added and stirred for additional 10 minutes. The solution was allowed to settle for 10 minutes, and a reddish-brown color solid was appeared and collected by vacuum filtration and washed with cold methanol (10 ml) and diethyl ether (10 mL). The desired product was collected as a fine reddish-brown powder. The brownish solid was dried in a vacuum oven overnight and characterized through NMR and HRMS. ^{1}H NMR (400 MHz, DMSO) δ 8.44 (d, *J* = 7.1 Hz, 1H), 7.71 (dddd, *J* = 20.8, 19.2, 10.9, 4.0 Hz, 4H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.48 (dd, *J* = 7.9, 1.5 Hz, 1H), 3.06 (t, *J* = 7.5 Hz, 2H), 2.88 (dd, *J* = 8.6, 6.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.88, 164.84, 142.06, 135.72, 133.56, 131.62, 130.91, 130.65, 129.59, 129.41, 129.34, 128.94, 128.30, 127.68, 126.81, 126.25, 125.86, 124.90, 26.06, 24.34. HRMS (ESI-QTOF) $(M\text{-}ClO^{-}{}_{4})^{+}$ obtained 361.1596, Calcd. 361.1587.

4. Synthesis of PY-S

An oven-dried round bottom flask was charged with 4-methylthio benzaldehyde (3.42 mmol) and α-Tetralone (6.84 mmol) in 10 ml Toluene and stirred at 110 ^{o}C for 30 minutes. Then perchloric acid (3.42 mmol) was added dropwise and the solution was stirred for one and a half hours. Black sticky precipitation was visible at the bottom of the flask. After completion of the reaction, the mixture was cooled down to room temperature and the toluene part was decanted so that only the black precipitation remains in the round bottom flask. This precipitation was dissolved in the minimum amount of methanol and diethyl ether (10 mL) was added and stirred for additional 10 minutes. The solution was allowed to settle for 10 minutes, and a reddish-brown color solid was collected by vacuum filtration and washed with cold methanol (10 ml) and diethyl ether (10 mL). The desired product was collected as a fine reddish-brown powder. The brownish solid was dried in a vacuum oven overnight and characterized through NMR and HRMS. ^{1}H NMR (400 MHz, DMSO) δ 8.42 (d, *J* = 7.7 Hz, 1H), 7.75 (td, *J* = 7.5, 1.2 Hz, 1H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.44 (d, *J* = 8.5 Hz, 1H), 3.05 (t, *J* = 7.4 Hz, 2H), 2.93 (dd, *J* = 8.4, 6.2 Hz, 2H), 2.59 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 165.16, 164.57, 142.80, 141.87, 135.63, 130.54, 129.48, 128.86, 128.82, 126.69, 126.19, 126.12, 26.15, 24.69, 14.55. $(M\text{-}ClO^{-}{}_{4})^{+}$ obtained 407.1468, Calcd. 407.1464.

5. Synthesis of PM-S

An oven-dried round bottom flask was charged with the previously synthesized **PY-S** (0.6507 mmol), methylamine (6.507 mmol, 40% in methanol), and methanol (5mL). The resulting solution was allowed to stir at room temperature for 10 minutes. After completion of the reaction, diethyl ether (5 mL) was added to the reaction mixture. The solution was allowed to settle for further 10 minutes, and the pale-yellow color solid was collected by vacuum filtration and washed with diethyl ether (10 mL). The desired product was collected as a fine pale-yellow powder. ^{1}H NMR (400 MHz, DMSO) δ 8.49 (d, *J* = 7.4 Hz, 1H), 7.86 – 7.42 (m, 4H), 7.34 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 1H), 2.91 – 2.82 (m, 2H), 2.65 – 2.57 (m, 2H), 2.56 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 152.78, 152.45, 142.65, 136.78, 132.99, 130.57, 129.69, 128.92, 128.37, 127.93, 127.46, 53.88, 43.80, 27.60, 26.90. $(M-ClO_4^-)^+$ obtained 420.1787, Calcd. 420.1780

6. Synthesis of PN-S

An oven-dried round bottom flask was charged with the previously synthesized **PY-S** (0.6507 mmol) and methanolic ammonia (6.507 mmol) and the resulting solution in methanol (5mL) was allowed to stir at room temperature for 2h. After completion of the reaction, diethyl ether (5 mL) was added to the reaction mixture. The solution was allowed to settle for 10 minutes, and the pale-yellow color solid was collected by vacuum filtration and washed with diethyl ether (10 mL). The desired product was collected as a fine pale-yellow powder. ^{1}H NMR (400 MHz, DMSO) δ 8.47 – 8.39 (m, 1H), 7.46 – 7.20 (m, 5H), 2.86 – 2.77 (m, 2H), 2.65 – 2.57 (m, 2H), 2.55 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 149.72, 141.16, 138.14, 138.14, 135.07, 129.75, 129.27, 128.16, 127.36, 126.28, 126.28, 125.16, 27.75, 27.67, 26.97, 25.79, 14.92, 14.70. $(M+H)^+$ obtained 406.1648, Calcd. 406.1624

7. Computational Methods.

All the computational calculations were performed using the Gaussian 09, Revision of D.01[1] quantum program software package installed at Magus High-Performance Cluster (HPC) computing facility of Shiv Nadar University (SNU). The geometrical structure of the electronic ground state was optimized by using the B3LYP[2] level of theory and 6-311++G (d, p)[3] basis set. The PCM[4] implicit solvation model using the integral equation formalism variant (IEFPCM)[5] was employed with water (Ɛ=78.3553) as the solvent which keywords demand that calculation will be performed in the presence of solvent by placing the solute in a cavity within the solvent reaction field. The ground state optimized structure of the molecules was confirmed to be global minima with no imaginary frequencies. For excited state calculation, the time-dependent density functional theorem (TD-DFT)[6-7] was employed. Vertical excitation (absorption) was calculated by the corrected linear response method (cLR)[8] by using the nonequilibrium solvation (PISALR)[8] model where electronic relaxation of the solvent polarization is taken into account at the same level of theory (keeping same the computational feasibility of linear response approaches). For the charge distributions on IndiFluors, NBO (Natural bonding orbital) calculation, version 3.1[9] installed in the Gaussian software package is done in the ground state using B3LYP/PCM level of theory with 6-311++G(d,p) basis set.

8. Photophysical studies

The steady-state photophysical properties of **PY-P**, **PY-S,** and **PM-S** were studied by UV-Vis and photoluminescence spectrophotometry. Firstly, 10 mM stock solutions of **PY-P**, **PY-S,** and **PM-S** were prepared in DMSO. Further, the stock solutions were diluted to the final experimental concentration in PBS buffer (10 mM, pH 7.4). All the spectroscopic studies such as absorption, solvent-dependent emission studies were performed utilizing HPLC grade solvents. A 10 mM stock solution in DMSO was also prepared for the solvent-dependent experiments and used in such a way so that the final DMSO concentration remains not more than 0.1% in respective solvents. 10 mM PBS buffer solution of pH 7.4 was measured as a blank solution before steady-state and the time-dependent UV-Vis kinetics studies. The time-dependent absorption study was conducted in the kinetics mode of the instrument and recorded the absorption wavelength in each 10 minutes interval for 3h. Steady-state emission spectra were evaluated at 445 and 375 nm excitation wavelengths. Concentration dependents experiments were performed similarly at different incubation times, with similar parameters.

9. Relative quantum yield measurements

Relative fluorescence quantum yields of the probe alone and probe in presence of HOCl were measured by taking PY as a reference 0.95. Quantum yields were calculated by using the following formula:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta^2{}_X}{\eta^2{}_{ST}}\right) \qquad \text{(Eq. 1)}$$

Where Φ_X is the quantum yield of the sample, Φ_{ST} is the quantum yield of the standard. $Grad_X$ and $Grad_{ST}$ are the gradients of the plot of the area under the curve of the emission spectra of samples and standards respectively and η represents the refractive index of respective solutions.

10. Limit of Detection calculation

The limit of detections (LOD) for the probes towards HOCl were calculated using Eq. 2:

$$\text{Limit of Detection (LOD)} = 3\sigma/\text{slope} \qquad \text{(Eq. 2)}$$

where σ standard deviation of 10 blank measurements and slope obtained from the graph of fluorescence intensity of the probe vs concentration of HOCl at lower concentrations.

11. Cell culture experiments

Human hepatocellular carcinoma cell line HepG2 was a generous gift from Dr. Deepak Sehgal's lab and HeLa from Dr. Koyeli Mapa's lab, Dept. of Life Sciences, Shiv Nadar University. Cells were maintained in DMEM with 10% FBS and 1% Pen-Strep under an atmosphere of 5% CO_2 at 37 ^{o}C. The biocompatibility of the probe was determined in terms of standard MTT [(3-(4,5-Dimethylthiazol-2-yl)-2-5- Diphenyl tetrazolium Bromide] assay before subjecting **PM-S** for the imaging experiments.

12. MTT Assay

Briefly, in 96 well plates, 10000 cells were incubated for 24 hrs followed by treatment of the adhered cells with the required concentrations of **PM-S** and washed after 2h and then incubated under similar environment for another 2, 6, 12, and 24 hrs. After incubation 10% final concentration of MTT solutions (As delivered and instructed by Hi-Media Laboratory, Mumbai, India) and incubated for 4 hr. with constant monitoring of the needle-shaped crystal appearance with a Leica microscope. After incubation media were removed carefully and 100 µl of solubilization buffer was added and further incubated for 30 minutes until complete dissolution of the crystals. After that absorbance was measured at 595 nm and the % of cell viability was calculated using the formula:

$$\text{Viability (\%)} = \text{Abs. sample}/\text{Abs. control} \times 100 \qquad \text{(Eq.3)}$$

13. Detection of Endogenous HOCl in HeLa cells in flow cytometry

5×10^5 HeLa cells were seeded in 35mm cell culture plate, next day when cells got attached, they were washed twice with 1X PBS followed by the treatment of **PM-S** in serum-free media for 30 minutes, followed by subsequent treatment with PMA and PMA+DPI in two plates. After treatment cells were trypsinized, harvested, washed then collected in mini centrifuged tubes. Finally, 50,000 cells were screened from each set of experiments in flow cytometer

CytoFLEX for quantitative analysis of the **PM-S** signal in presence of inducer (PMA) and inducer with inhibitor (PMA+DPI) in the DAPI channel. Prior to those unstained cells were loaded to set the voltage and of DAPI, SSC and FSC.

14. 96 Well plate Assay

HepG2 cells were seeded in 96-well microplates at a density of $1x10^5$ cells/mL one day before the assay in DMEM (Dulbecco's modified Eagle medium; high glucose) supplemented with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin. All reagents and probe solutions were freshly dissolved in fresh media (DMEM). Before experiment, cells were washed with PBS and then co-incubated the probe in presence or absence of inducers or inhibitors for 30 minutes. At the end of 30-min coincubation, all reagents were removed, and cells were washed two times with PBS before fluorescence reading on the microplate reader (excitation at 375 nm and emission at 490 nm). Data was collected by a area scanning method and 20 highest intensity points were taken into consideration for quantification.

15. Normalized absorbance and emission spectra of PY, PY-P, and PY-S

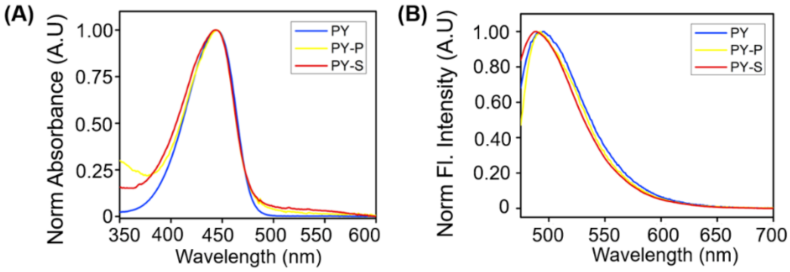


Fig. S1 (A) Normalized absorbance spectra (B) Normalized emission spectra of **PY**, **PY-P,** and **PY-S** in PBS (10 mM, pH 7.4, containing 0.1% DMSO).

16. Solvent Dependent Studies

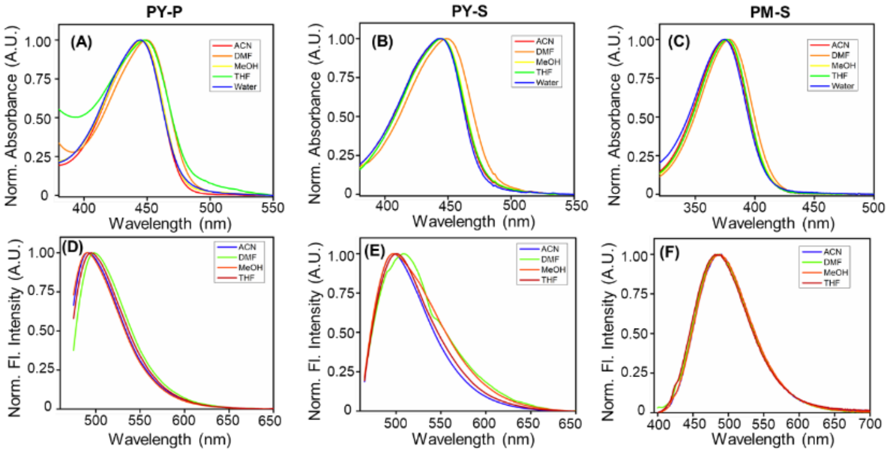


Fig. S2 (A, B, C) Normalized absorbance spectra of **PY-P**, **PY-S**, **PM-S** (10 μM) in different solvents (containing 0.1% DMSO); (D, E, F) Normalized fluorescence emission spectra of **PY-P**, **PY-S**, **PM-S** (10 μM) in different solvents (containing 0.1% DMSO).

17. Orthogonality of the core scaffold and benzene receptor moiety

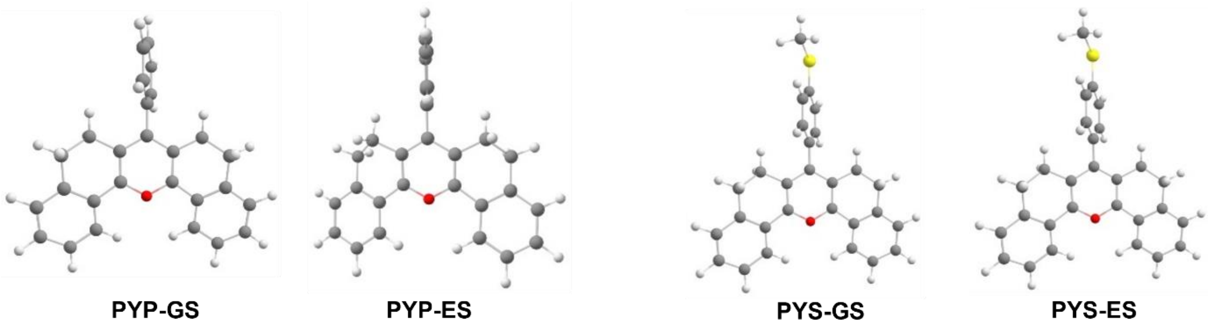


Fig. S3 Optimized ground state and excited state structure of **PY-P** and **PY-S**. (GS= Ground state, ES= Excited-state).

18. FMO of oxidized forms of PY-S and PM-S

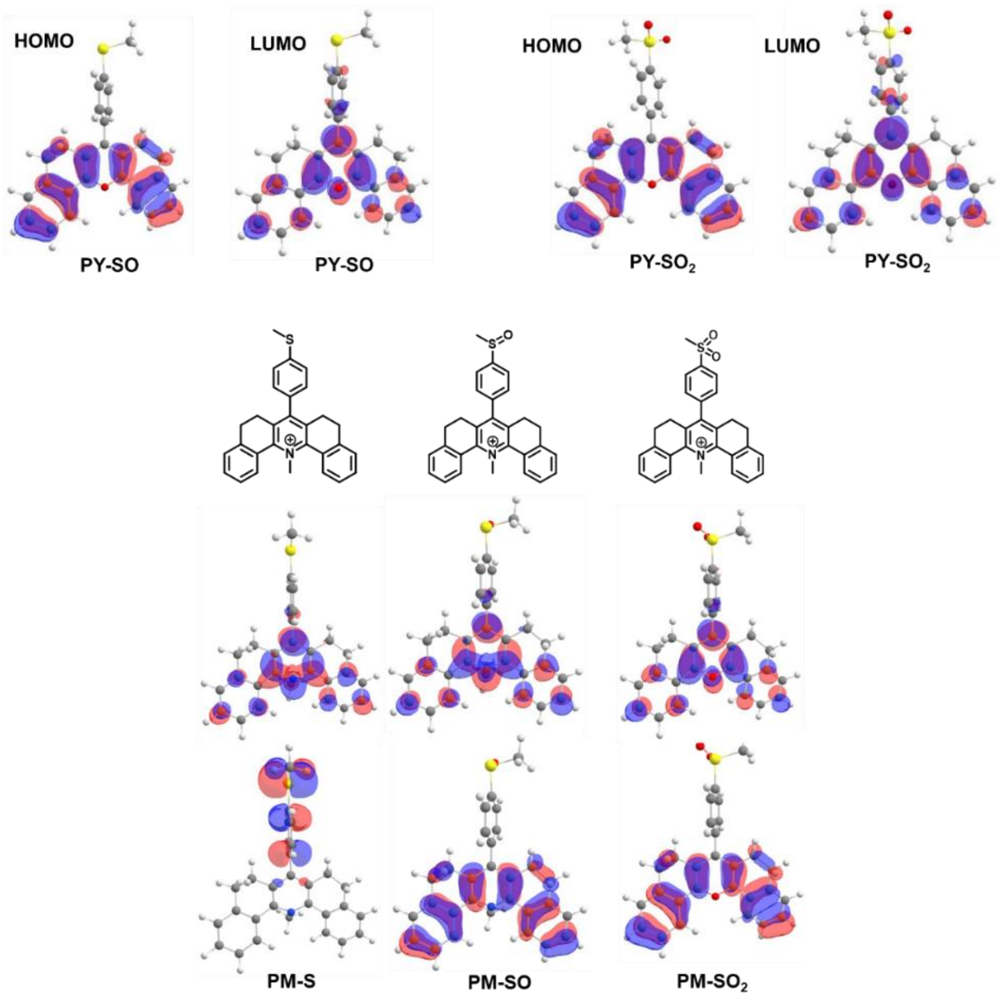


Fig. S4 FMO of both the single and double oxidized form of **PY-S and PM-S**

19. Table S1: for oscillator strengths of un-oxidized and oxidized and forms of PY-S & PM-S

	Exp		B3LYP		
Dye	**λabs (nm)**	**State**	**λabs (nm)**	**f (E)**	**Transitions**
PY-S	445	S1 S2	537.50 435.57	0.1411 0.5744	H-L (H-1)-L
PY-SO		S1	437.60	0.5902	H-L
$PY\text{-}SO_2$		S1	439.69	0.5807	H-L
PM-S	375	S1 S2	426.45 364.51	0.1295 0.5245	(H-1)-L (H-1)-L H-(L+1)
PM-SO		S1	366.31	0.5466	H-L
$PM\text{-}SO_2$		S1	368.24	0.5430	H-L

20. Emission spectra of PY-S in presence of different ROS

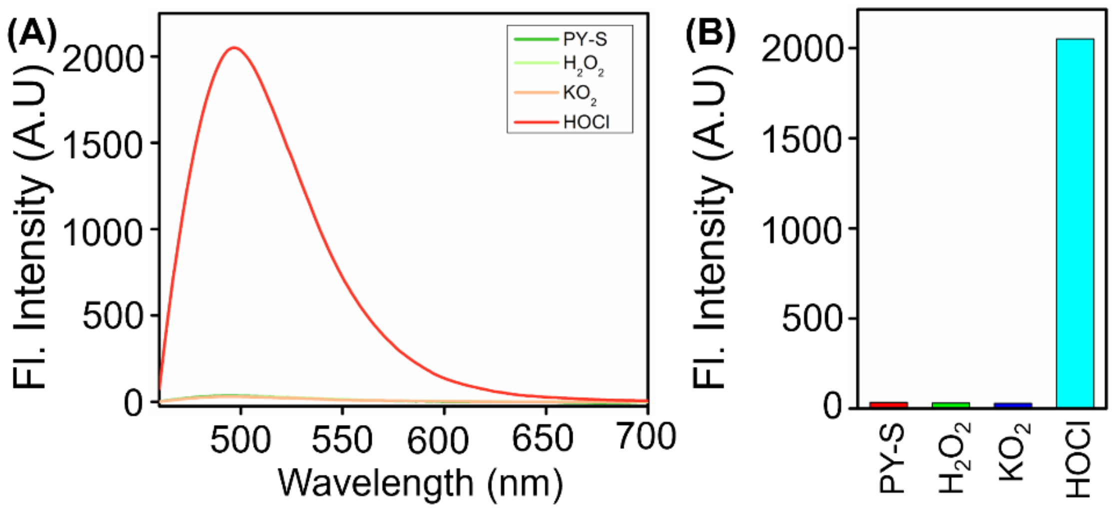


Fig S5. Fluorescence emission spectra of **PY-S** alone and in presence of HOCl (100 µM) and KO_2 and H_2O_2 (1mM) in PBS (pH 7.4, containing 0.1% DMSO), under excitation at 445 nm.

21. Fold of enhancement of PY-S and PM-S

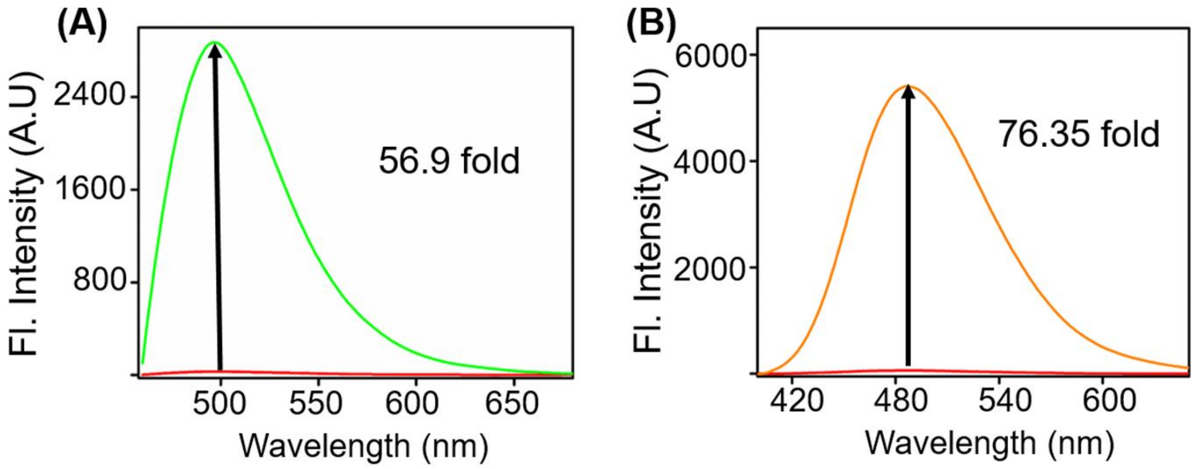


Fig. S6 Fold of enhancement of (A) **PY-S** and (B) **PM-S** upon reaction with HOCl.

22. Natural Bond Orbital (NBO) Calculation of PY-S, PM-S, and PN-S

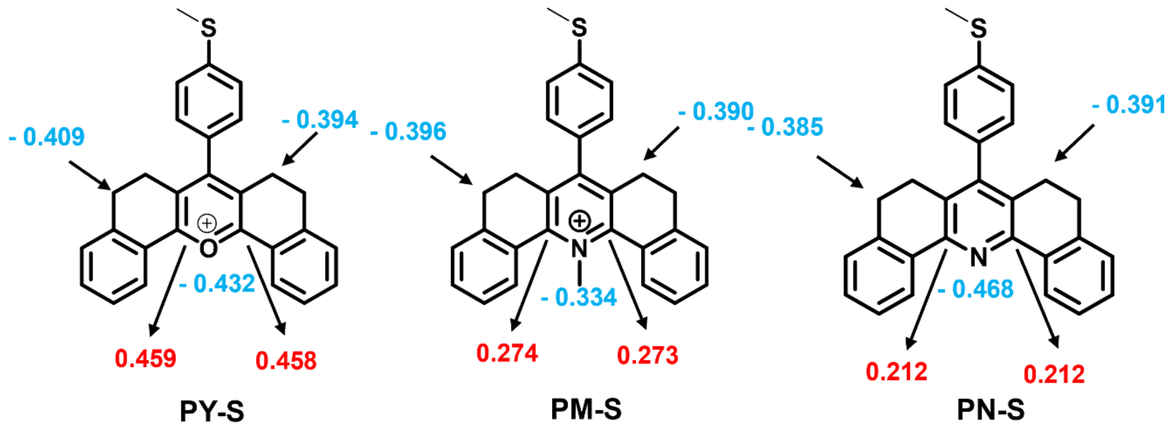


Fig. S7 Natural Bond Orbital (NBO) Calculation of **PY-S**, **PM-S,** and **PN-S** using density functional theory (DFT) with the hybrid B3LYP level of theory and 6-311++G (d, p) basis set.

23. Solubility Study of PM-S

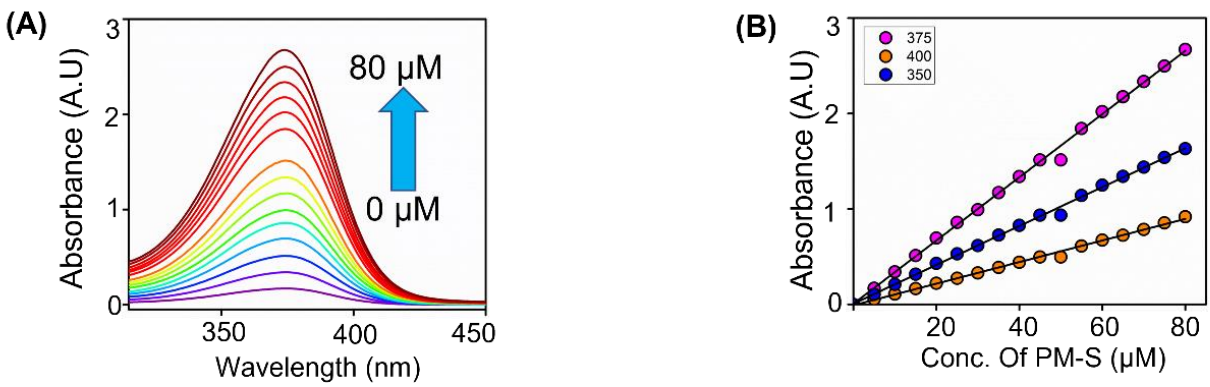


Fig. S8 Absorbance spectra of **PM-S** at different concentrations (0–80 μM) in PBS (pH 7.4, containing 0.1% DMSO).

24. Selectivity studies of PM-S in presence of different bio analytes

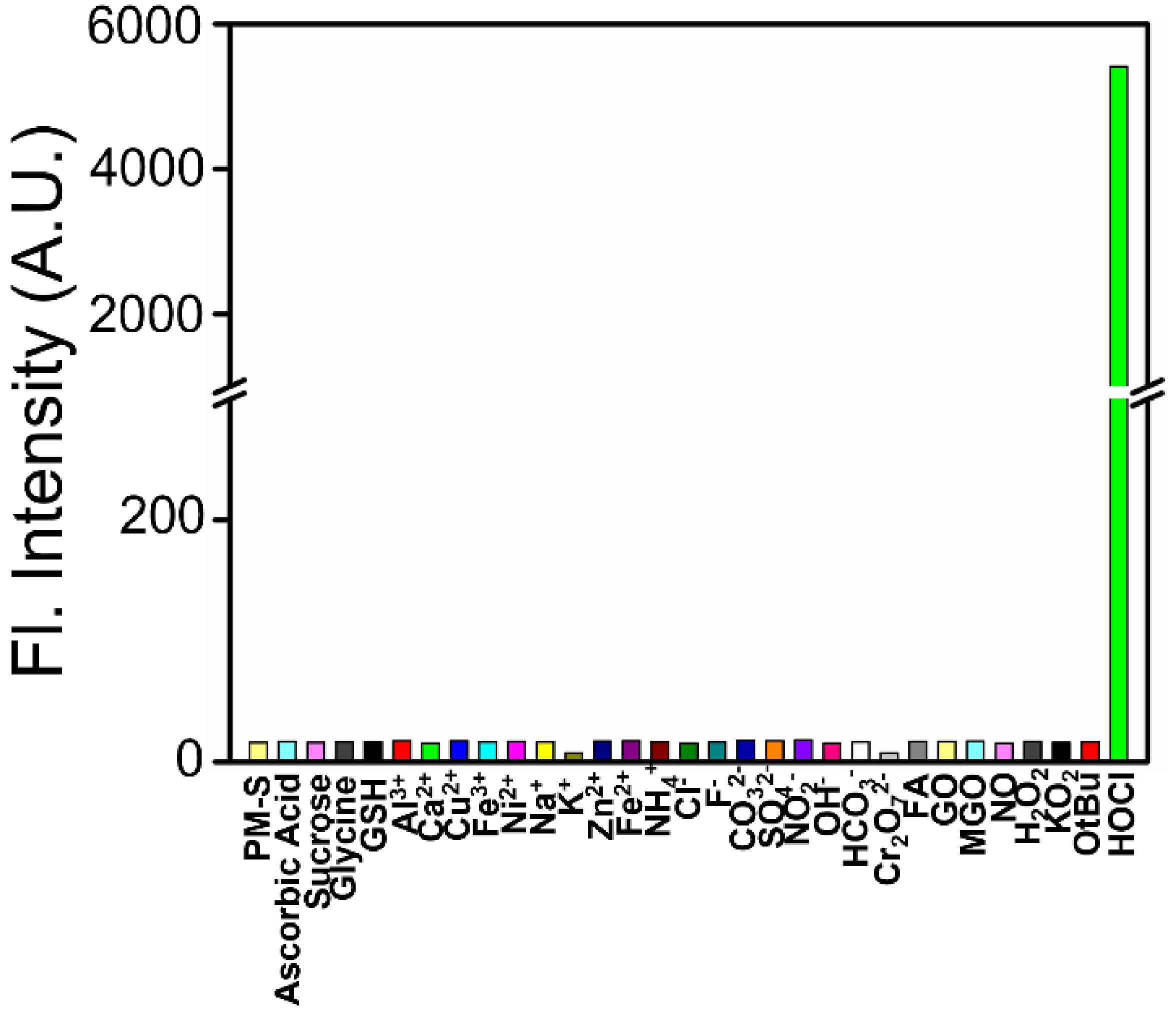


Fig. S9 Fluorescence emissive response of **PM-S** alone and in presence of another probable interfering biologically relevant analytes in PBS (pH 7.4, containing 0.1% DMSO) under excitation at 375 nm.

25. Advantages of PM-S over PY-S

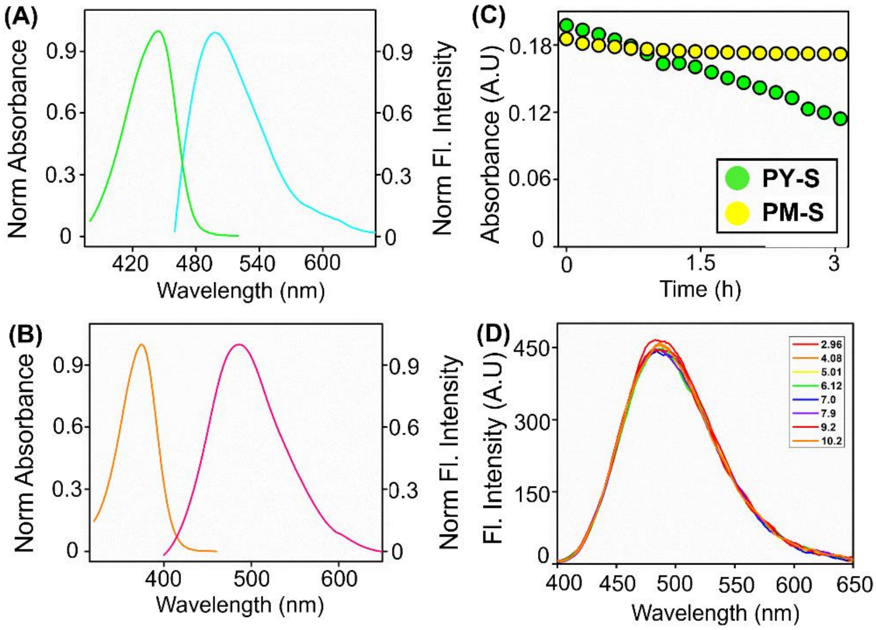


Fig. S10 (A) Normalized absorbance and emission spectra of **PY-S** (10 μM) in PBS (pH 7.4, containing 0.1% DMSO). (B) Normalized absorbance and emission spectra of **PM-S** (10 μM) in PBS (pH 7.4, containing 0.1% DMSO). (C) Time-dependent absorbance spectra of **PY-S** (green) and **PM-S** (yellow) with constant UV light irradiation for 3h. (D) Fluorescence emission spectra of **PM-S** at different pH (3-10) under excitation at 375 nm in PBS (pH 7.4, containing 0.1% DMSO).

26. High-Performance Liquid Chromatography (HPLC)

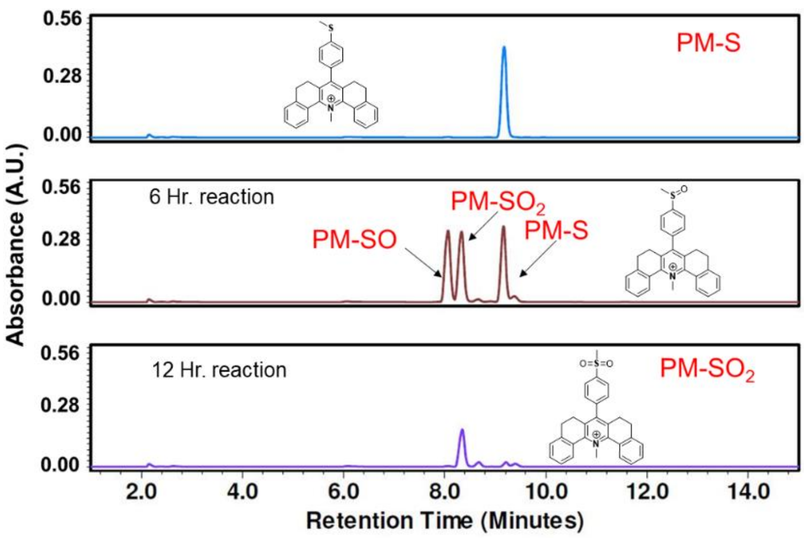


Fig S11 High-Performance Liquid Chromatogram of **PM-S**. **PM-SO**, **PM-SO$_2$.**

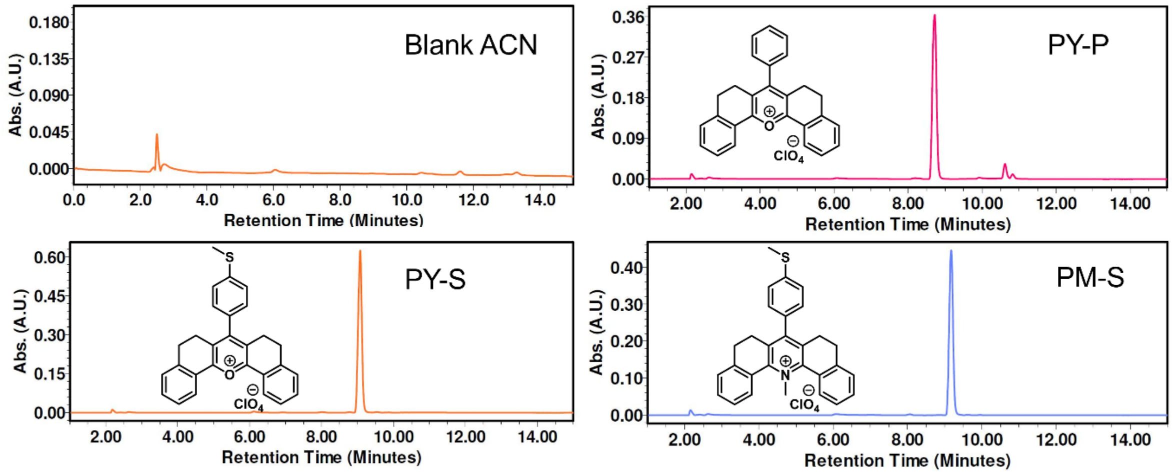


Fig S12 High-Performance Liquid Chromatogram of **PY-P**, **PY-S**, and **PM-S** confirming the purity of the molecules.

27. Cytotoxicity Study of PM-S

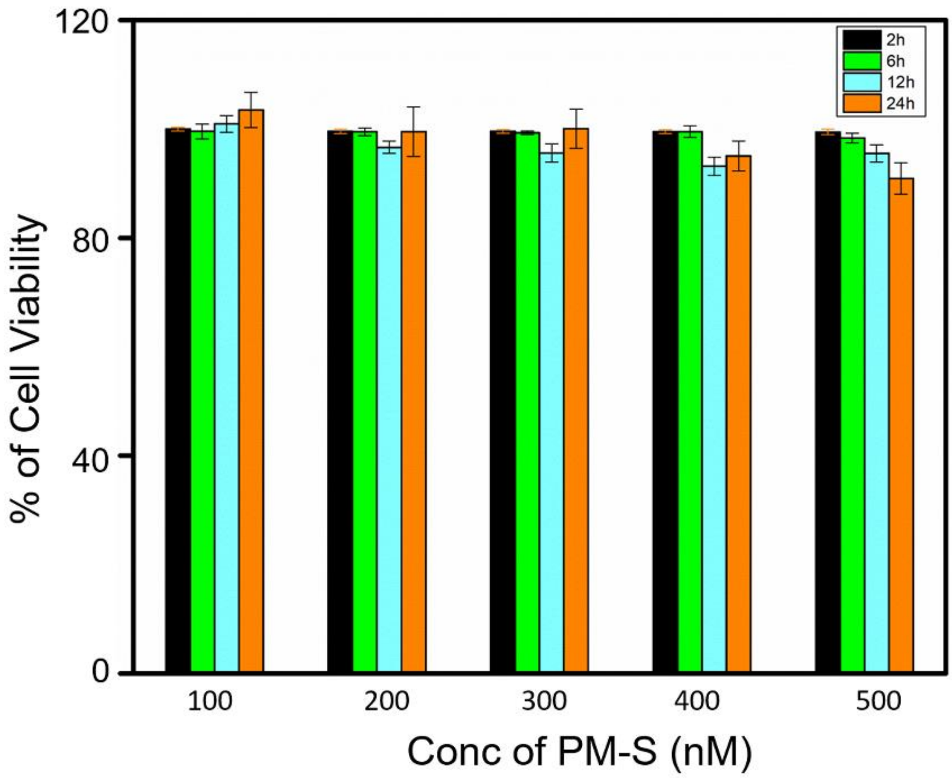


Fig S13. Biocompatibility study of **PM-S** calculated by standard MTT assays.

28. Bioimaging experiments of PM-S

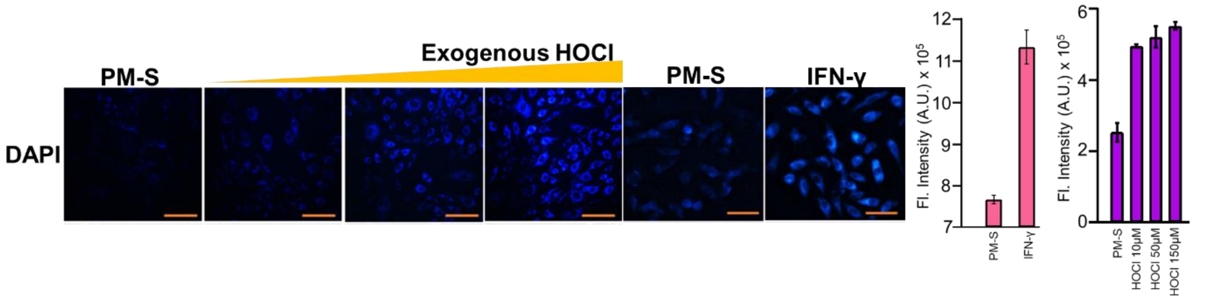


Fig. S14 HepG2 cells were incubated with **PM-S** (500 nM) in the presence of different concentrations of HOCl (0-150 μM) for 15 min, before fluorescence microscopy imaging. HeLa cells were incubated with **PM-S** (500 nM) in the presence or absence of IFN-γ (5 ng/mL) 30 min before fluorescence microscopy imaging. The scale bar represents 70 μm.

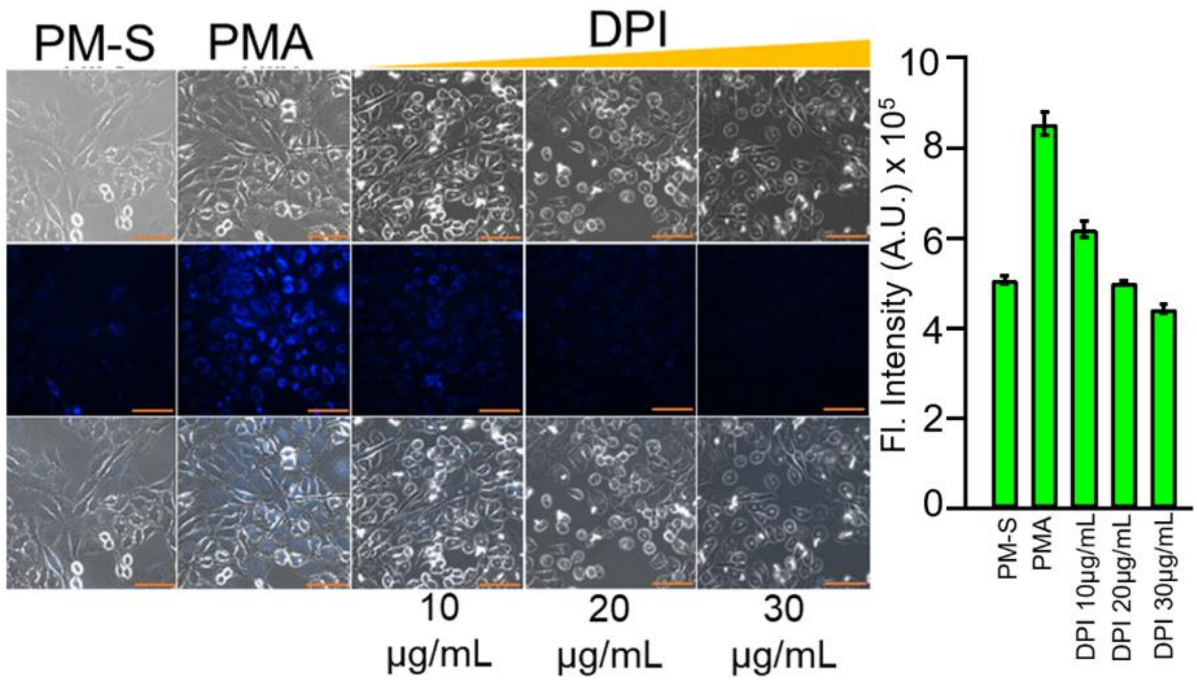


Fig. S15 HepG2 cells were incubated with **PM-S** (500 nM) in the presence or absence of PMA (200 ng/mL), PMA plus DPI (10, 20, or 30 μg/mL) for 30 min, before fluorescence microscopy imaging. The scale bar represents 70 μm.

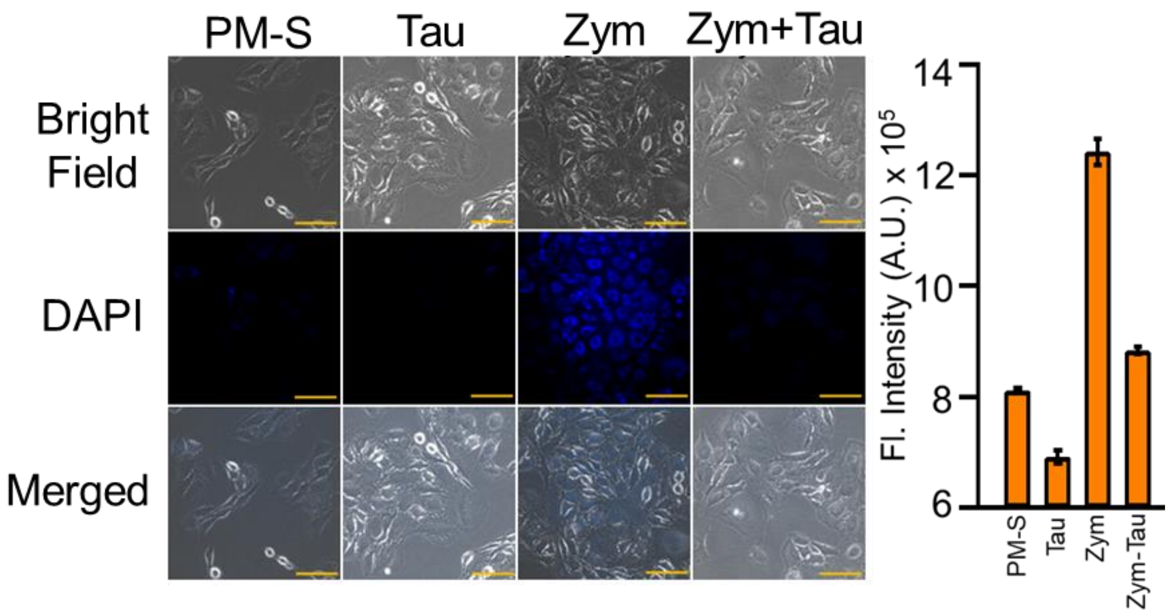


Fig. S16 HepG2 cells were incubated with **PM-S** (500 nM) in the presence or absence of zymosan (10 µg/mL) or taurine (20 mM) or zymosan plus taurine (10 mM) for 30 min, before fluorescence microscopy imaging. Scale bar represents 70 µm.

29. Localization experiment of PM-S in oxidative stress condition

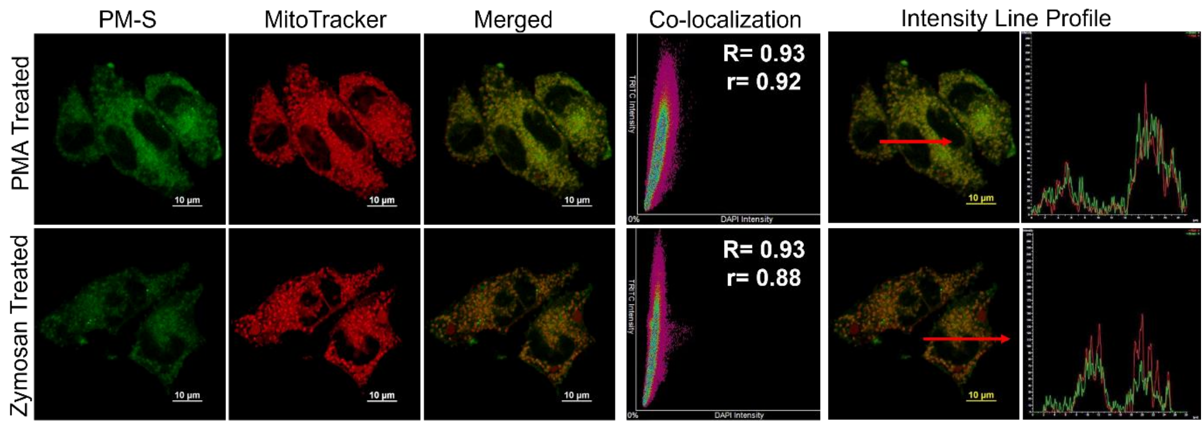


Fig S17 Cells were incubated with **PM-S** (500 nM) in the presence of PMA (200 ng/mL) and Zymosan (500 ng/mL) for 30 min, along with Mito-tracker red (200 nM) for 15 minutes in each set of experiment, before confocal microscopy imaging.

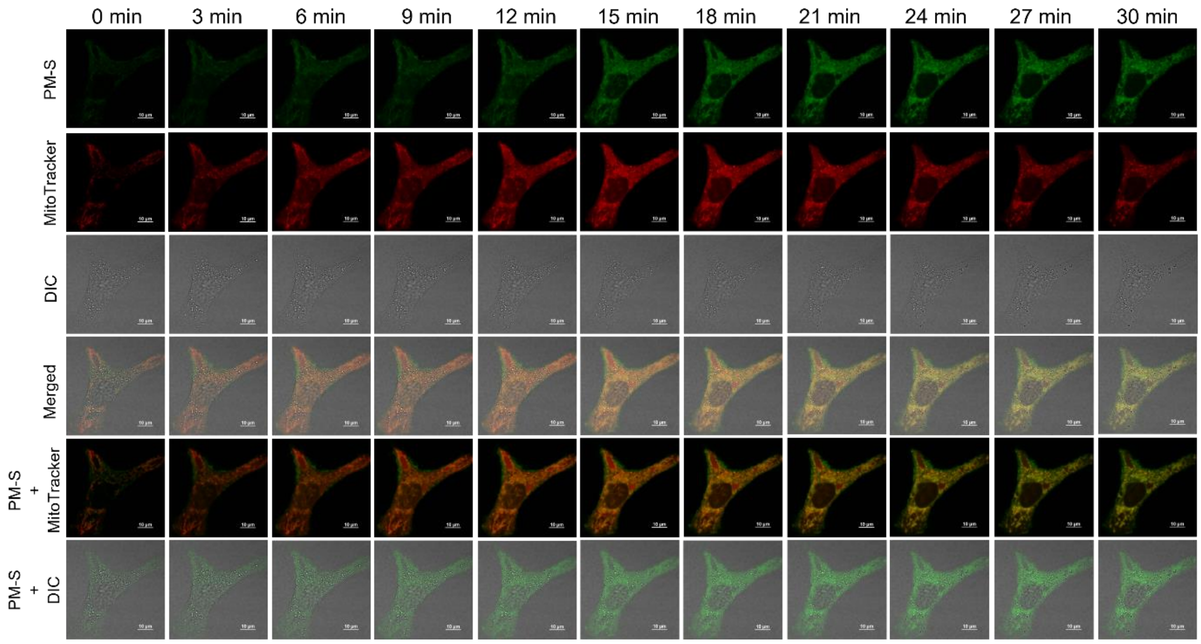


Fig S18 Time-lapse confocal images of **PM-S** (500 nM) in the presence of PMA (200 ng/mL) and Mito-tracker (200 nM) from 0-30 minutes.

30. Flow Cytometry Analysis

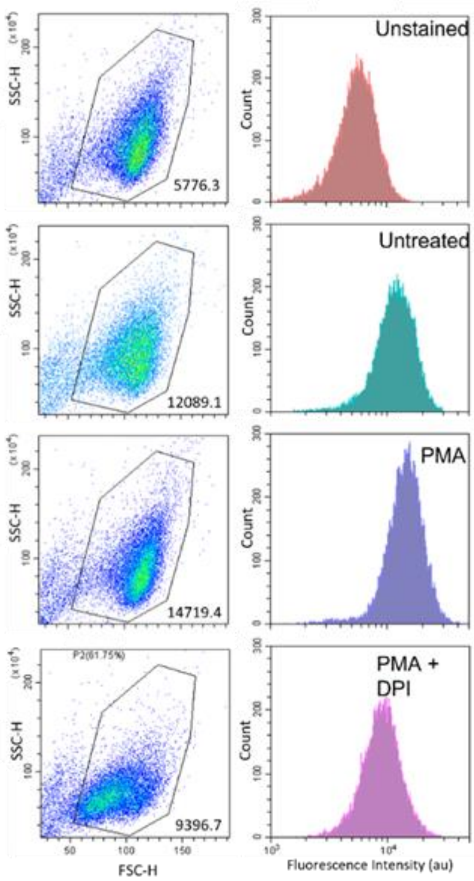


Fig. S19 Flow Cytometric Analysis for detection of endogenous HOCl in HeLa cells.

31. Relative quantum yield of PY-S and PM-S before and after addition of HOCl

	Φ_f		Φ_f
PY-S	0.01	**PM-S**	0.01
PY-S + HOCl	0.31	**PM-S + HOCl**	0.42

32. Mass-spectrometric study

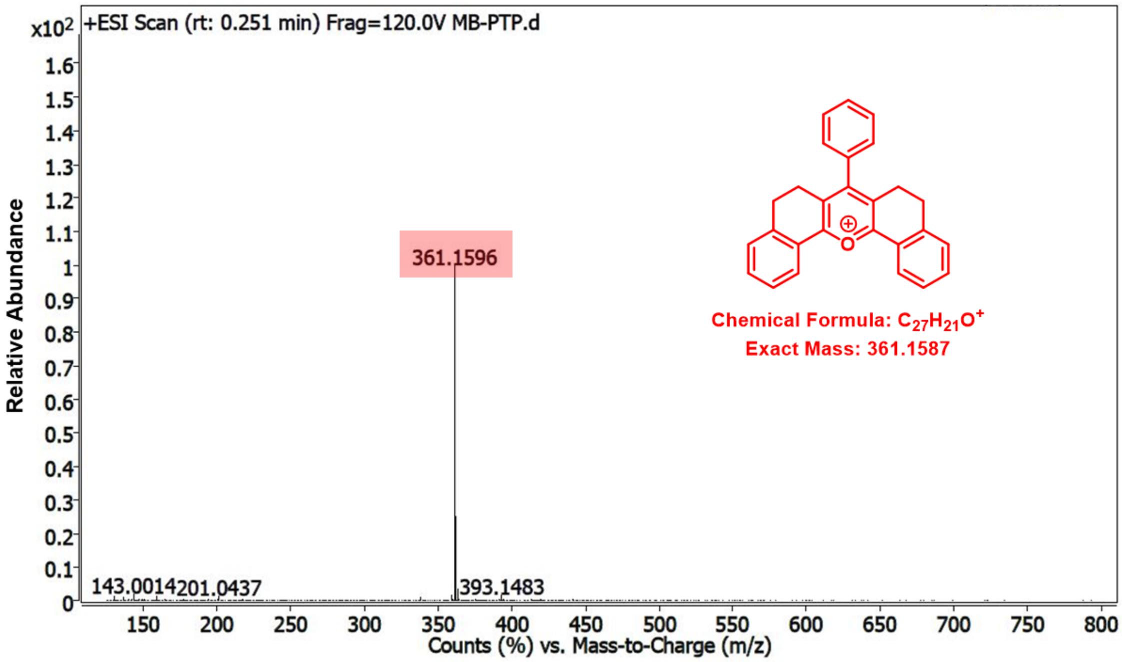


Fig. S20 ESI-HRMS study of **PY-P.**

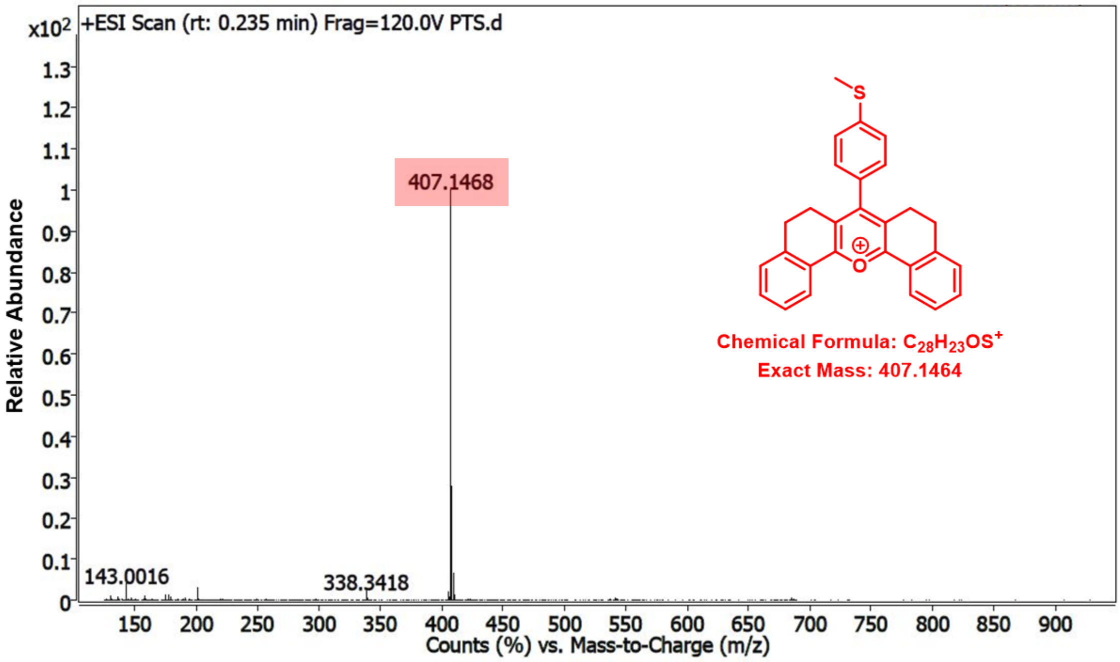


Fig. S21 ESI-HRMS study of **PY-S.**

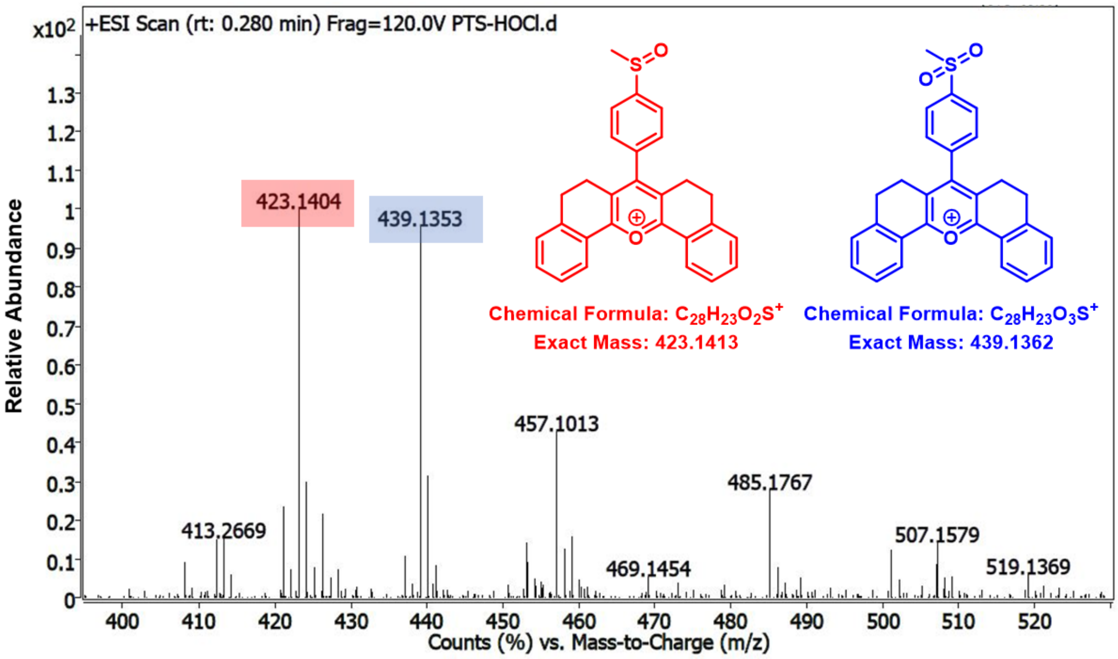


Fig. S22 ESI-HRMS study of **PY-S** in presence of HOCl.

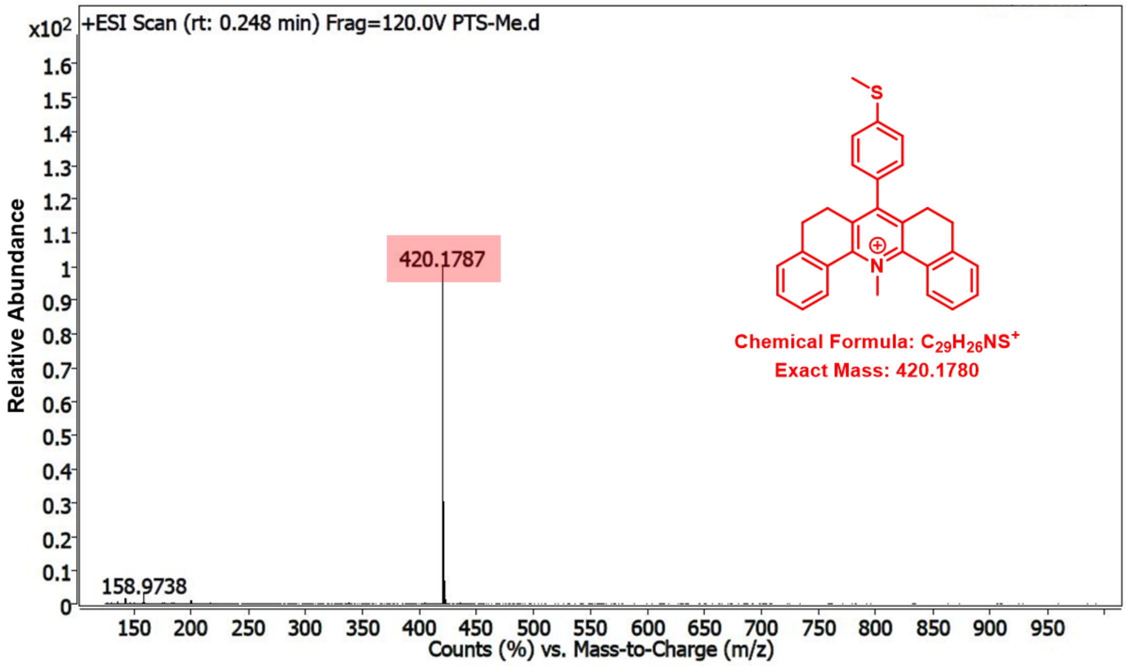


Fig. S23 ESI-HRMS study of **PM-S.**

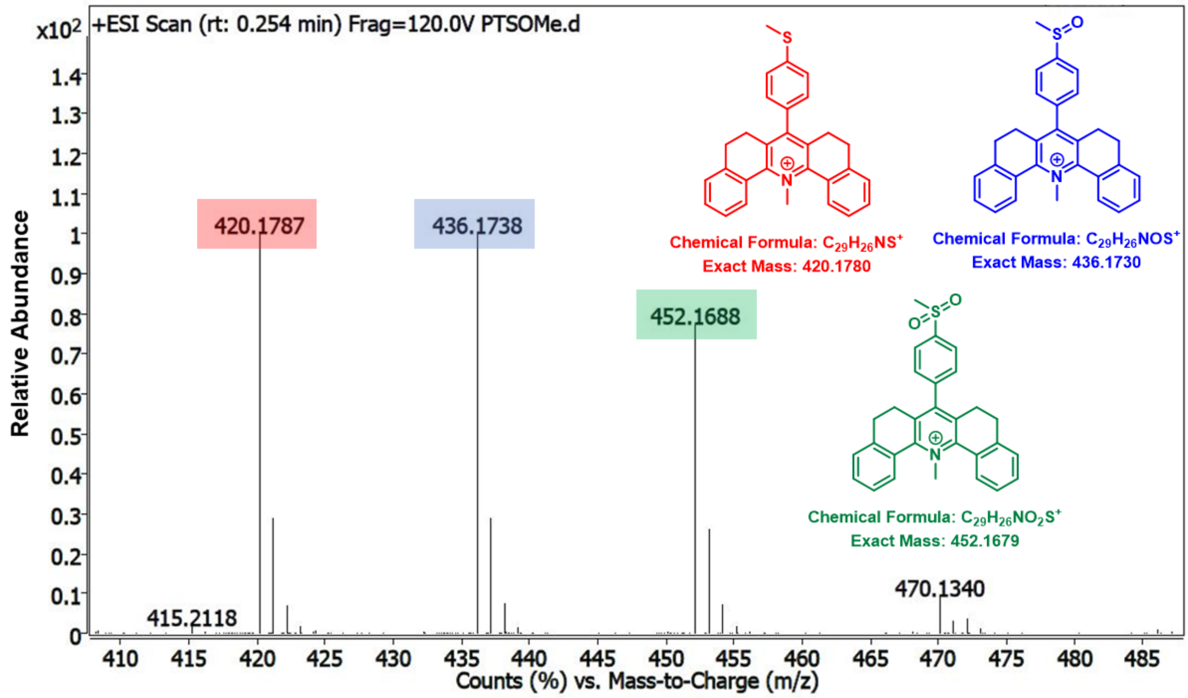


Fig. S24 ESI-HRMS study of **PM-S** in presence of HOCl.

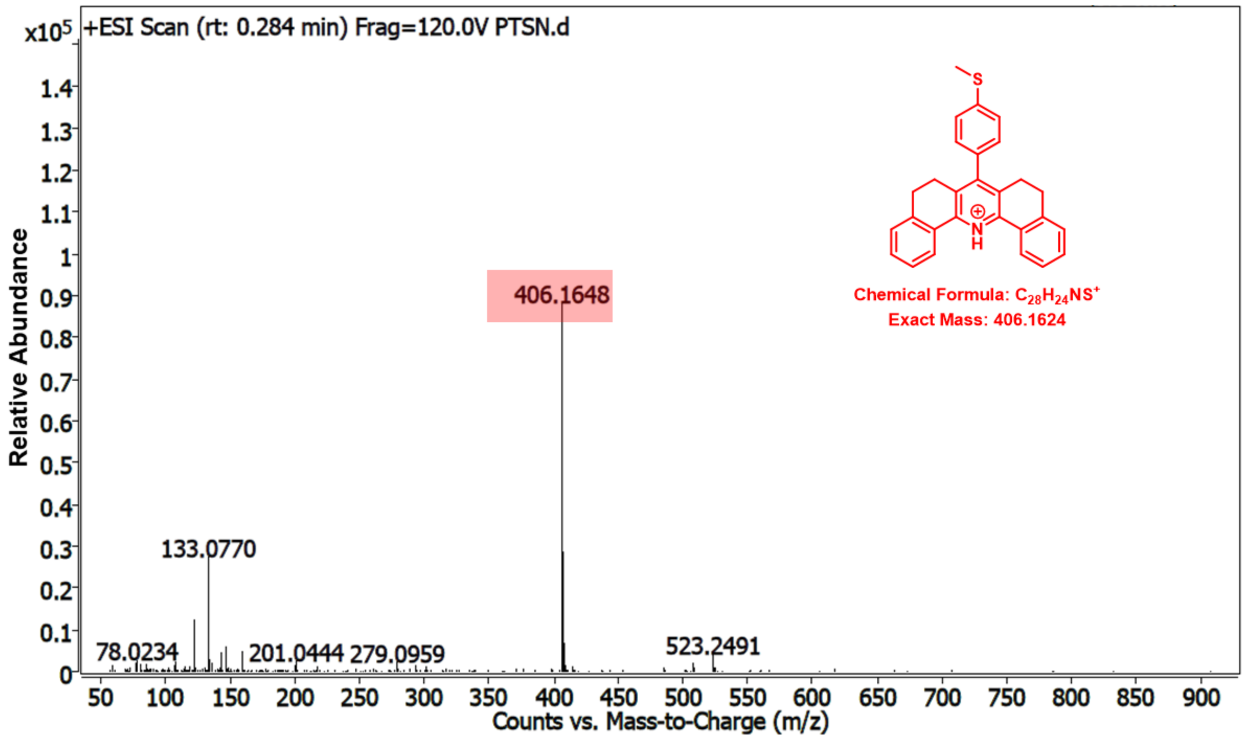


Fig. S25 ESI-HRMS study of **PN-S.**

33. Nuclear Magnetic Resonance (NMR) spectroscopy

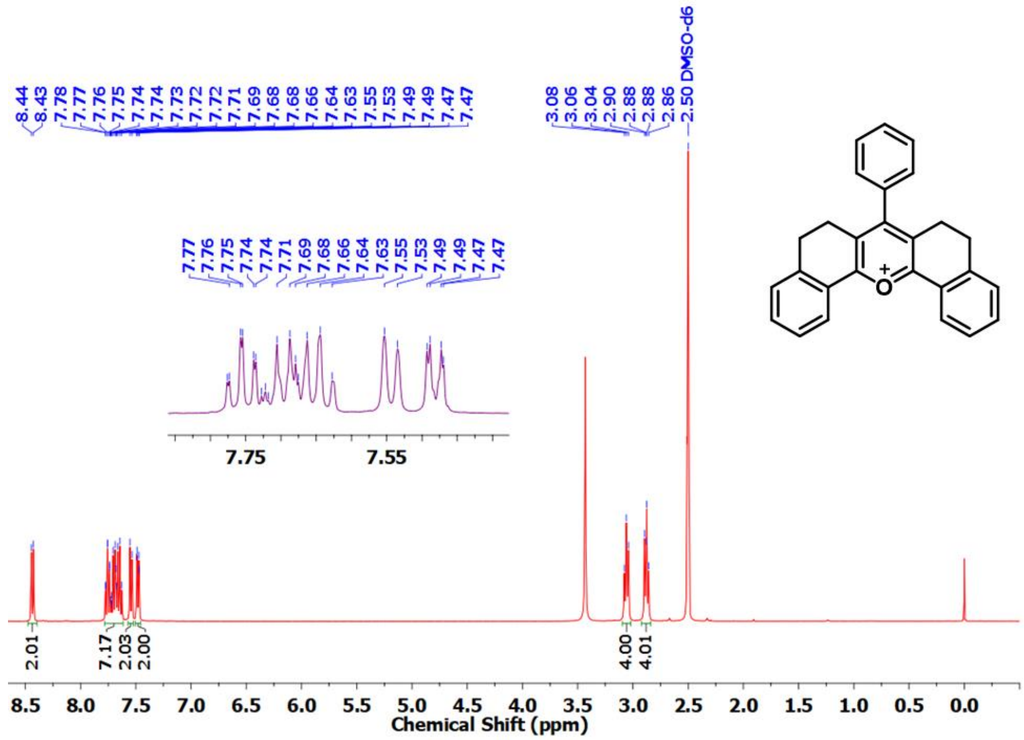


Fig. S26: ^{1}H NMR of **PY-P**.

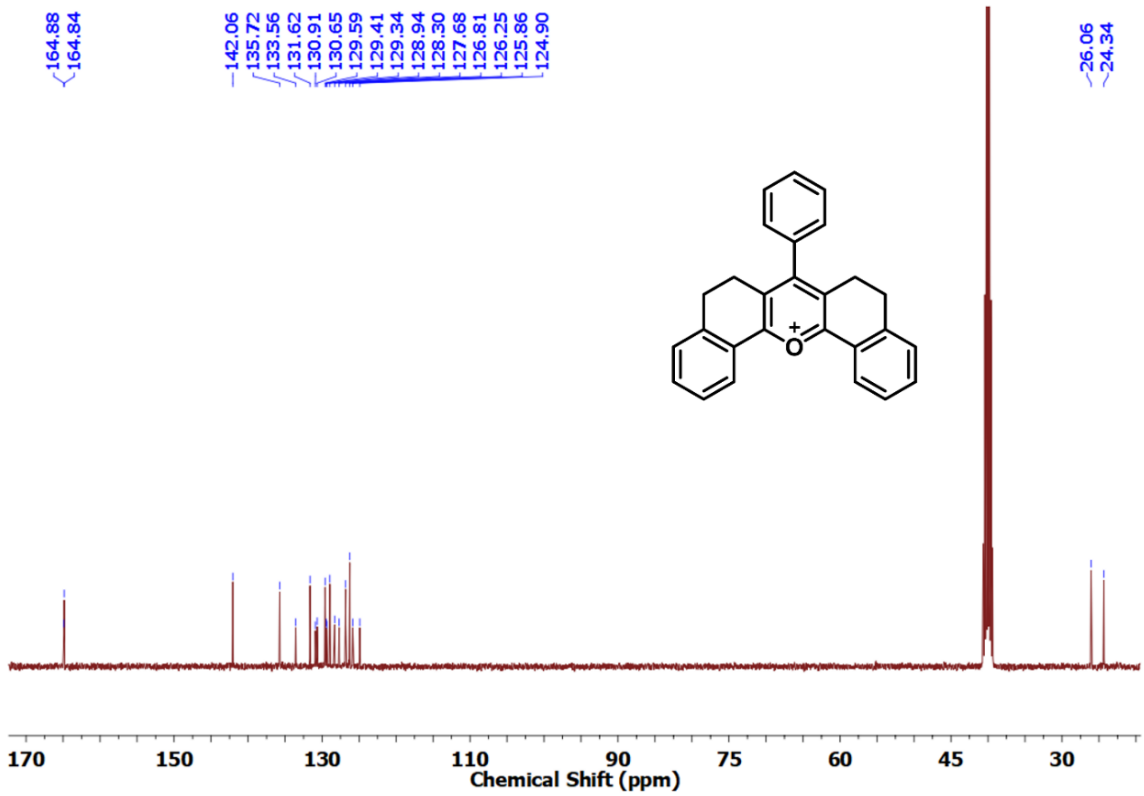


Fig. S27: ^{13}C NMR of **PY-P**.

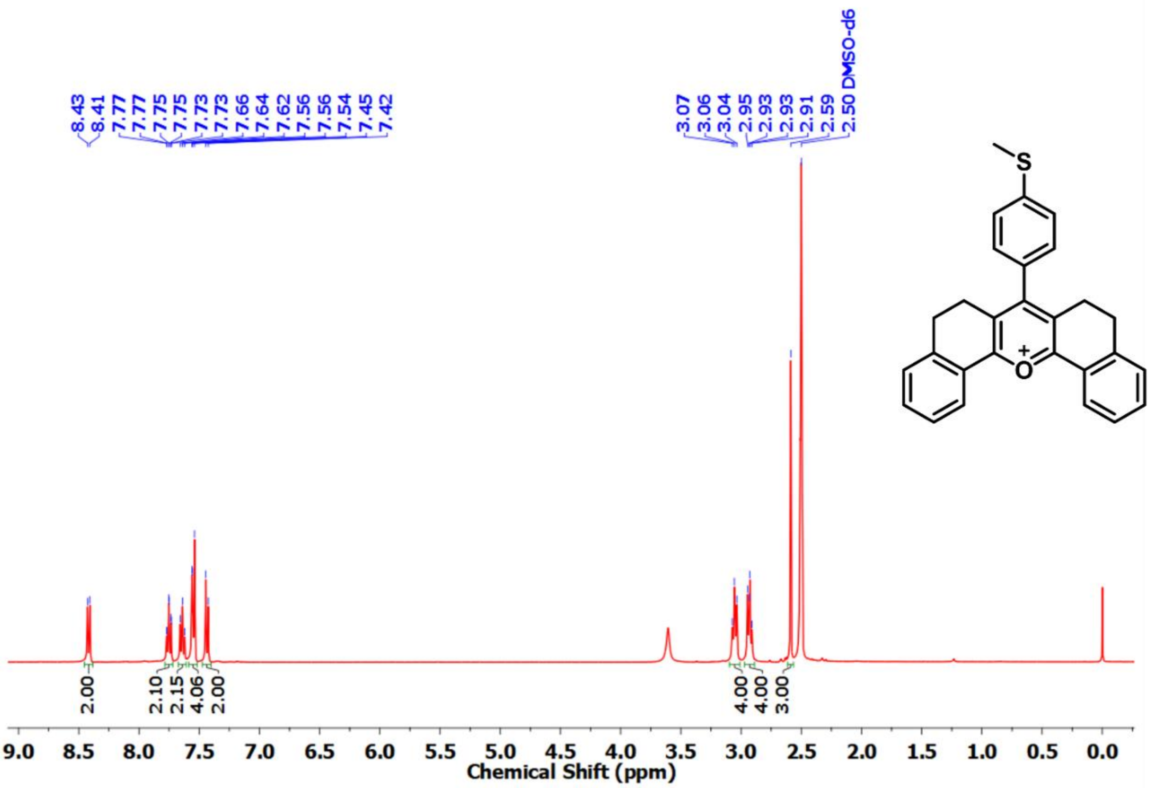


Fig. S28: ^{1}H NMR of **PY-S**.

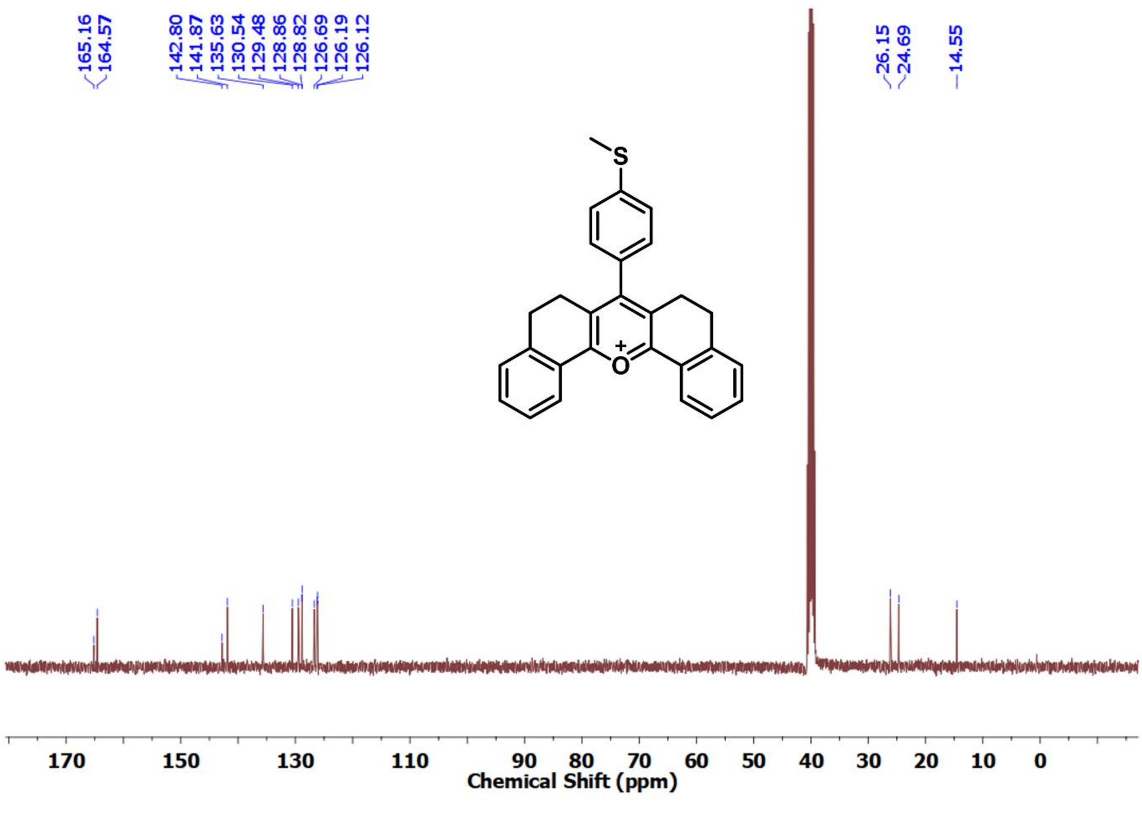


Fig. S29: ^{13}C NMR of **PY-S**.

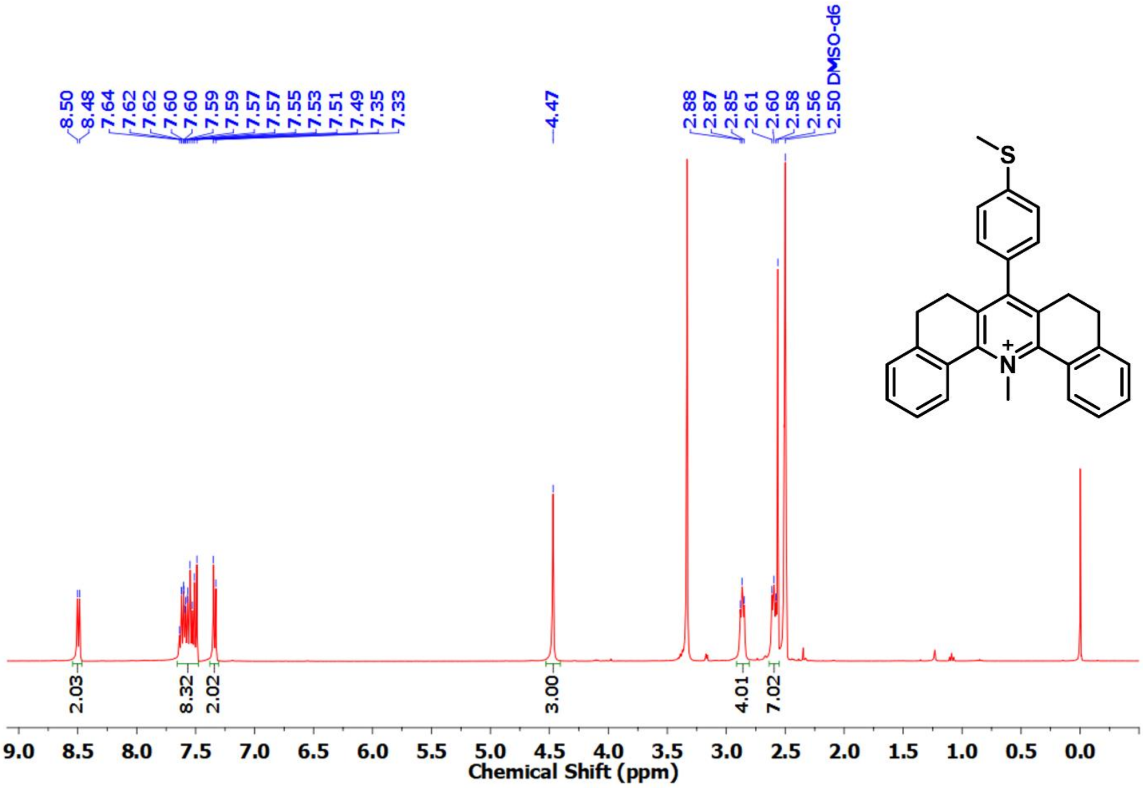


Fig. S30: ^{1}H NMR of **PM-S**.

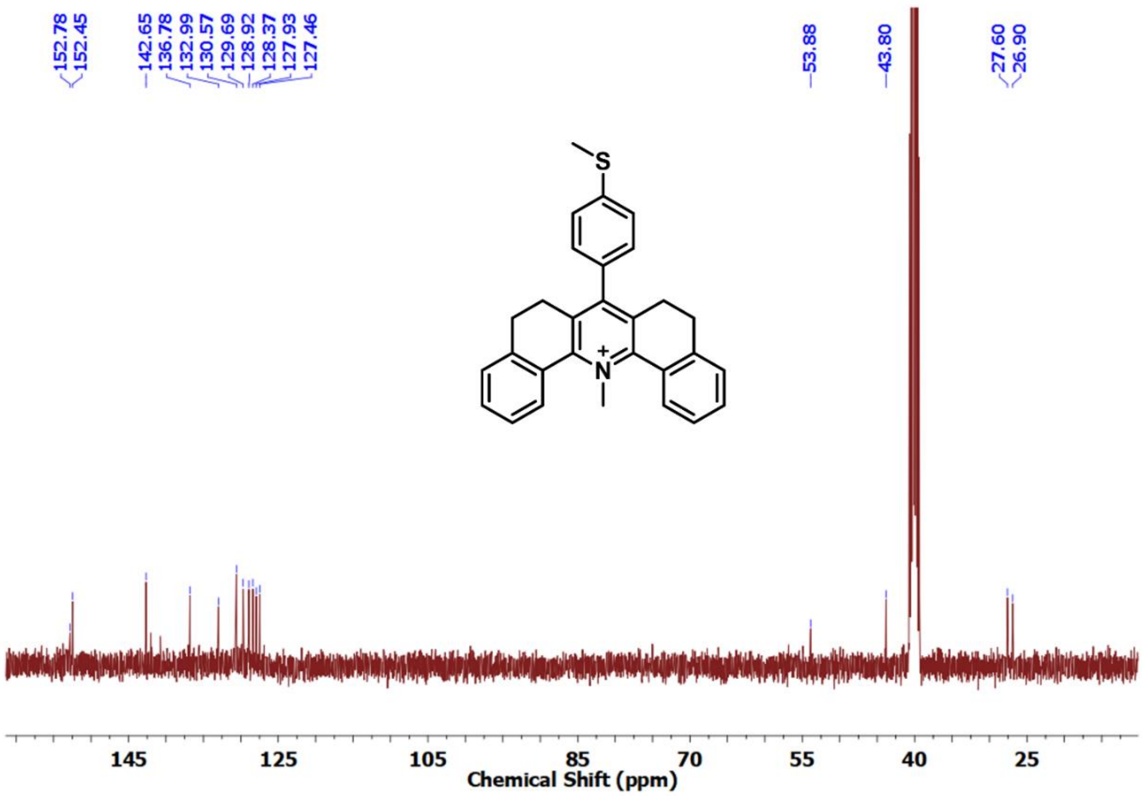


Fig. S31: ^{13}C NMR of **PM-S**.

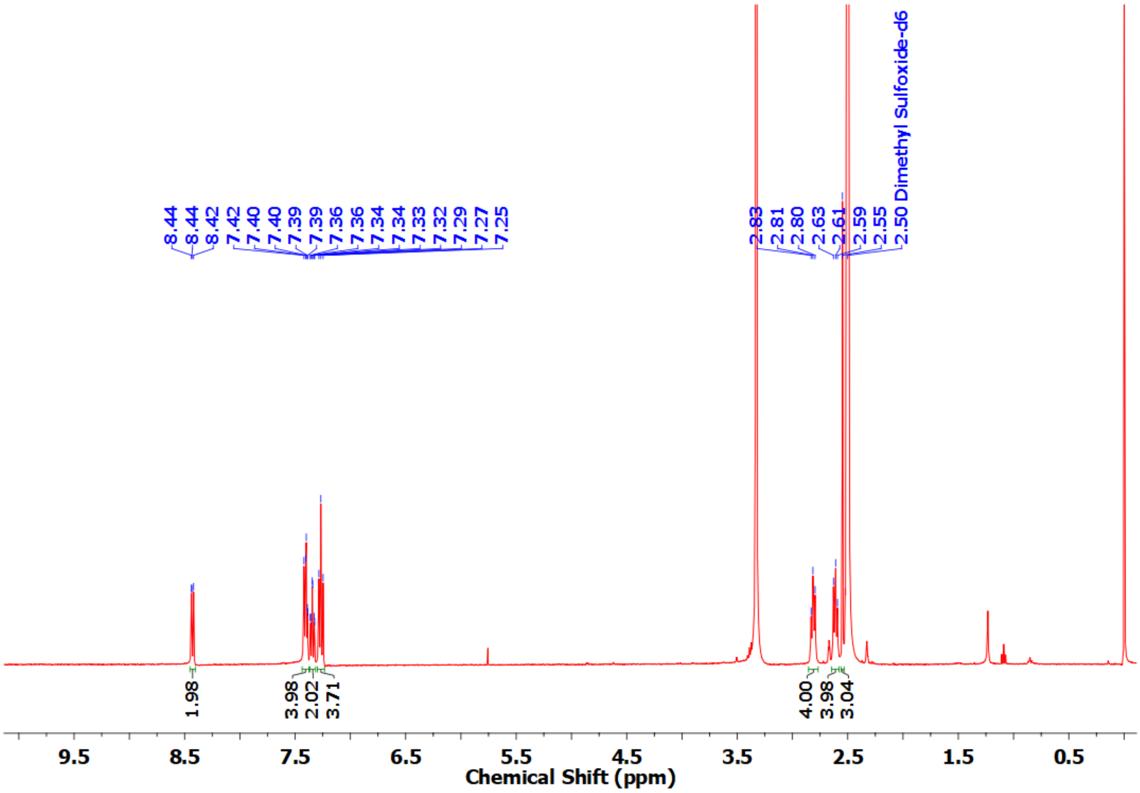


Fig. S32: ^{1}H NMR of **PN-S**.

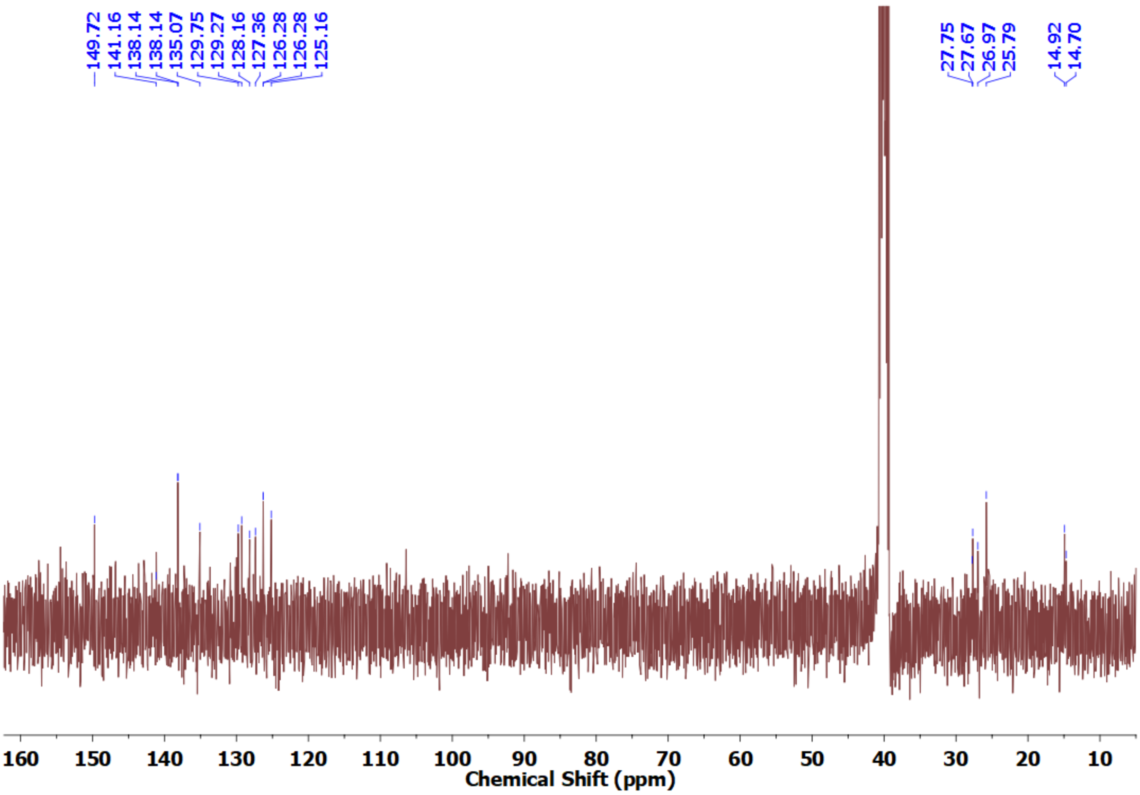
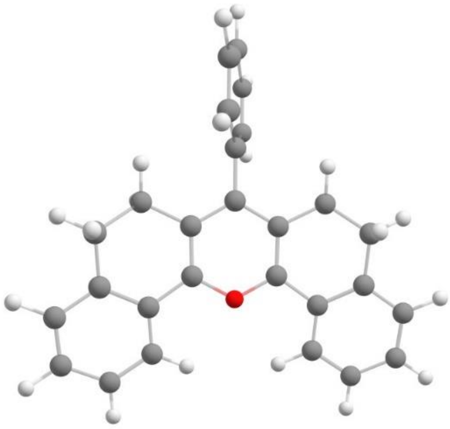


Fig. S33: ^{13}C NMR of **PN-S**.

34. Coordinates of Theoretical Calculations

S34 Ground state PYP



6	1.149465000	0.735901000	0.023454000
6	1.257979000	-0.647472000	0.011864000
6	-1.081719000	-0.902343000	0.005938000
6	-1.272735000	0.471381000	0.025003000
6	-0.138372000	1.306840000	0.042148000
6	-2.163796000	-1.870537000	0.002356000
6	-3.457356000	-1.402547000	-0.318819000
6	-3.642512000	0.044321000	-0.706380000
6	-2.697385000	0.972758000	0.063657000
6	2.434152000	1.527092000	0.105736000
6	3.575444000	0.828052000	-0.639571000
6	3.693200000	-0.627246000	-0.257873000
6	2.522102000	-1.361526000	0.034104000
6	-1.950298000	-3.226646000	0.302978000
6	-3.016368000	-4.114337000	0.293491000
6	-4.298634000	-3.655974000	-0.019872000
6	-4.512678000	-2.312476000	-0.324408000
6	4.918393000	-1.290902000	-0.237929000
6	4.989368000	-2.650137000	0.063093000
6	3.827666000	-3.372736000	0.347772000
6	2.596804000	-2.733203000	0.332016000
6	-0.306154000	2.787903000	0.102096000
6	-0.666409000	3.403969000	1.306003000
6	-0.822160000	4.788059000	1.363307000
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6	-0.280878000	4.951312000	-0.982926000
6	-0.112036000	3.569370000	-1.043443000
1	-3.452425000	0.144570000	-1.782644000
1	-4.677159000	0.349204000	-0.541472000
1	-2.747532000	1.983452000	-0.340470000

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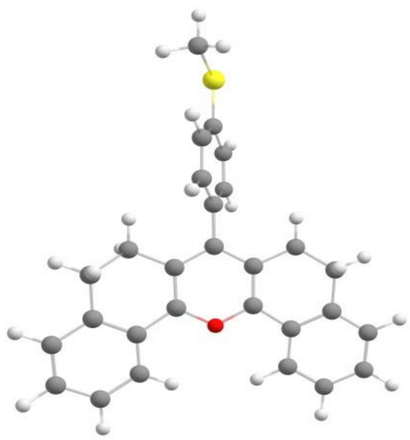
1	2.286615000	2.531791000	-0.286425000
1	2.695124000	1.637603000	1.166094000
1	3.396321000	0.893017000	-1.720463000
1	4.516530000	1.345468000	-0.446377000
1	-0.957239000	-3.574404000	0.554509000
1	-2.853209000	-5.157859000	0.533578000
1	-5.133509000	-4.347150000	-0.024279000
1	-5.510454000	-1.966887000	-0.570668000
1	5.824718000	-0.739233000	-0.461318000
1	5.952424000	-3.147188000	0.078567000
1	3.885642000	-4.427901000	0.585404000
1	1.695849000	-3.286445000	0.561260000
1	-0.817710000	2.805361000	2.196906000
1	-0.135715000	5.547923000	-1.876021000
8	0.157678000	-1.414242000	-0.000984000
1	-0.758754000	6.638720000	0.265741000
1	-1.093928000	5.257711000	2.301502000
1	0.160597000	3.097784000	-1.980755000

S35 Ground State PY-S

6	0.227522000	1.212629000	0.057485000
6	1.614024000	1.193880000	0.021462000
6	1.655309000	-1.158465000	0.003089000
6	0.271440000	-1.225001000	0.048670000
6	-0.462014000	-0.018817000	0.082586000
6	2.523347000	-2.323252000	-0.029756000
6	1.937422000	-3.564710000	-0.361155000
6	0.474657000	-3.611702000	-0.728182000
6	-0.352701000	-2.601577000	0.073728000
6	-0.431355000	2.568113000	0.182922000
6	0.349336000	3.647546000	-0.572633000



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6	3.897272000	-2.236791000	0.252082000
6	4.685475000	-3.378140000	0.214886000
6	4.109832000	-4.609404000	-0.108710000
6	2.748419000	-4.697258000	-0.395831000
6	2.594585000	4.781337000	-0.215540000
6	3.960779000	4.721347000	0.054277000
6	4.575898000	3.494050000	0.315090000
6	3.822272000	2.329419000	0.306289000
6	-1.945818000	-0.053989000	0.164326000
6	-2.584939000	-0.553785000	1.308494000
6	-3.969525000	-0.586223000	1.389003000
6	-4.757863000	-0.140414000	0.315919000
6	-4.124346000	0.346498000	-0.833227000
6	-2.734313000	0.396703000	-0.899472000
6	-7.188002000	0.397319000	-1.048825000
1	0.375775000	-3.393148000	-1.799169000
1	0.079505000	-4.617458000	-0.576130000
1	-1.369628000	-2.555125000	-0.314742000
1	-0.427640000	-2.932255000	1.116866000
1	-1.459394000	2.531436000	-0.169752000
1	-0.473992000	2.820492000	1.250470000
1	0.238848000	3.487280000	-1.652855000
1	-0.072793000	4.630501000	-0.356949000
1	4.337438000	-1.283235000	0.511372000
1	5.742662000	-3.312462000	0.441307000
1	4.723153000	-5.502662000	-0.134815000
1	2.310861000	-5.655932000	-0.650923000
1	2.125557000	5.737380000	-0.419467000
1	4.547144000	5.632848000	0.064376000
1	5.636821000	3.449696000	0.528904000

1	4.292674000	1.378516000	0.517090000
1	-1.998369000	-0.905053000	2.149394000
1	-4.441507000	-0.963203000	2.289375000
1	-4.697795000	0.690066000	-1.683213000
1	-2.265021000	0.773112000	-1.801365000
1	-8.270939000	0.339215000	-0.941016000
1	-6.879938000	-0.222848000	-1.890160000
1	-6.901361000	1.436269000	-1.209114000
16	-6.520613000	-0.239704000	0.522729000
8	2.278629000	0.028876000	-0.010009000

S36 Ground State PY-SO

6	-0.418478000	1.181666000	-0.004427000
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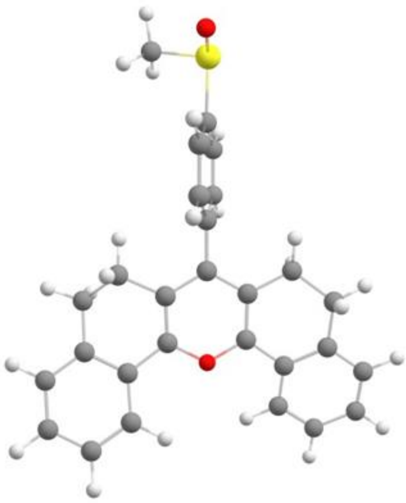
6	-1.946225000	-1.126648000	-0.016204000
6	-0.563712000	-1.250807000	-0.029702000
6	0.211782000	-0.076416000	-0.034950000
6	-2.860063000	-2.253871000	-0.043550000
6	-2.329633000	-3.532416000	0.240042000
6	-0.874694000	-3.661626000	0.619850000
6	0.005237000	-2.647298000	-0.117670000
6	0.316956000	2.500581000	-0.036662000
6	-0.442426000	3.587740000	0.731152000
6	-1.898588000	3.648438000	0.338976000
6	-2.577453000	2.452529000	0.014721000
6	-4.225770000	-2.097916000	-0.337876000
6	-5.060862000	-3.205207000	-0.358265000
6	-4.540573000	-4.472352000	-0.081691000
6	-3.187843000	-4.629942000	0.215642000
6	-2.617524000	4.842008000	0.343790000
6	-3.976935000	4.857980000	0.035290000
6	-4.644310000	3.671785000	-0.281298000



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6	1.701457000	-0.159791000	-0.098596000
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6	3.743558000	-0.103373000	-1.397012000
6	4.471828000	-0.301251000	-0.226125000
6	3.838357000	-0.450381000	1.001467000
6	2.448241000	-0.378713000	1.063847000
6	6.674166000	1.328588000	-0.485583000
1	-0.780499000	-3.504575000	1.701912000
1	-0.521584000	-4.674264000	0.418630000
1	1.019218000	-2.668249000	0.279119000
1	0.075869000	-2.917973000	-1.178866000
1	1.319562000	2.379800000	0.372646000
1	0.437823000	2.801230000	-1.084881000
1	-0.379197000	3.382712000	1.807430000
1	0.032284000	4.556659000	0.568512000
1	-4.622109000	-1.116347000	-0.560800000
1	-6.111448000	-3.085889000	-0.593135000
1	-5.190628000	-5.339396000	-0.100782000
1	-2.794071000	-5.616559000	0.432505000
1	-2.109320000	5.766846000	0.592663000
1	-4.517330000	5.797509000	0.039003000
1	-5.699639000	3.687402000	-0.524602000
1	-4.459016000	1.551593000	-0.544343000
1	1.782941000	0.137181000	-2.234871000
1	4.240318000	-0.015010000	-2.356741000
1	4.429678000	-0.625548000	1.892258000
1	1.947386000	-0.490298000	2.018518000
1	7.760233000	1.407307000	-0.525510000
1	6.274735000	1.864571000	0.375060000
1	6.236963000	1.689889000	-1.416550000
16	6.290345000	-0.451826000	-0.304645000

8	-2.516773000	0.086763000	0.005605000
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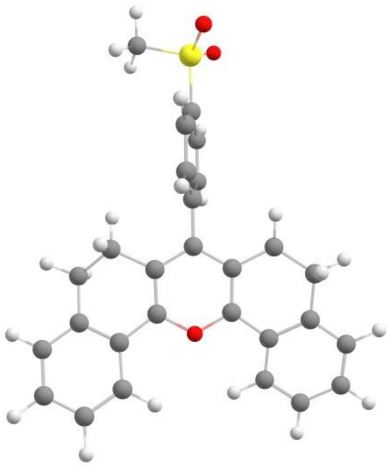
S37 Ground State PY-SO_2



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6	0.748294000	-1.230075000	0.018251000
6	0.018099000	-0.026454000	-0.009589000
6	3.007044000	-2.314272000	0.077214000
6	2.440118000	-3.572906000	-0.221896000
6	0.993664000	-3.646211000	-0.646076000
6	0.127506000	-2.606767000	0.074541000
6	0.014579000	2.555853000	-0.093986000
6	0.827990000	3.632793000	0.633003000
6	2.279997000	3.622855000	0.221739000
6	2.904392000	2.390185000	-0.070871000
6	4.367388000	-2.203819000	0.413428000
6	5.161906000	-3.340052000	0.459140000
6	4.605933000	-4.588363000	0.166525000
6	3.257968000	-4.699710000	-0.172011000
6	3.047963000	4.784476000	0.178357000
6	4.402660000	4.732200000	-0.147369000
6	5.015631000	3.509331000	-0.433423000
6	4.271377000	2.339327000	-0.394126000
1	0.937794000	-3.476743000	-1.728813000
1	0.597730000	-4.646636000	-0.464754000
1	-0.871751000	-2.585993000	-0.359501000
1	0.009564000	-2.889882000	1.127844000
1	-0.107071000	2.832779000	-1.148514000
1	-0.986373000	2.490898000	0.331704000
1	0.390274000	4.614885000	0.447393000

1	0.770363000	3.461560000	1.715395000
1	4.787665000	-1.235305000	0.651355000
1	6.208126000	-3.258052000	0.727135000
1	2.837092000	-5.672271000	-0.401000000
1	2.582561000	5.737706000	0.402449000
1	6.066980000	3.473140000	-0.691297000
1	4.736008000	1.390040000	-0.627064000
6	-1.474129000	-0.059463000	-0.014695000
6	-2.170951000	-0.395386000	1.151577000
6	-2.178513000	0.240224000	-1.186279000
6	-3.562096000	-0.425414000	1.152511000
1	-1.631184000	-0.628664000	2.061292000
6	-3.569610000	0.202449000	-1.196415000
1	-1.644591000	0.490003000	-2.095059000
6	-4.244706000	-0.127290000	-0.024039000
1	-4.104206000	-0.689028000	2.051528000
1	-4.117567000	0.416547000	-2.104947000
16	-6.048184000	-0.167658000	-0.028988000
6	-6.554869000	1.497102000	0.433770000
1	-6.163445000	2.195616000	-0.303591000
1	-7.644963000	1.493052000	0.426822000
1	-6.172989000	1.711879000	1.430248000
1	5.224417000	-5.477545000	0.205956000
1	4.981971000	5.647596000	-0.181889000
8	-6.492021000	-0.416349000	-1.413266000
8	2.749578000	0.033497000	0.002424000
8	-6.484130000	-1.094313000	1.032341000

S38 Ground State PM-S

6	-0.319782000	-1.192185000	0.087548000
6	-1.711538000	-1.121557000	-0.074589000
6	-1.565686000	1.266912000	-0.119277000
6	-0.176497000	1.194342000	-0.265929000
6	0.461964000	-0.045070000	-0.115058000
6	-2.162458000	2.603353000	0.115399000
6	-1.564410000	3.699856000	-0.538709000
6	-0.337525000	3.433204000	-1.369730000
6	0.567777000	2.471799000	-0.587347000
6	0.280547000	-2.514072000	0.505556000
6	-0.694121000	-3.235649000	1.442504000
6	-1.983851000	-3.448616000	0.696071000
6	-2.469035000	-2.402147000	-0.123850000
6	-3.165246000	2.837928000	1.071260000
6	-3.635525000	4.126419000	1.298700000
6	-3.102415000	5.199222000	0.584486000
6	-2.059345000	4.983857000	-0.313864000
6	-2.648946000	-4.673099000	0.719273000
6	-3.754958000	-4.904237000	-0.094948000
6	-4.180637000	-3.909573000	-0.971764000
6	-3.543637000	-2.672969000	-0.988199000
1	-0.606655000	2.980970000	-2.331723000
1	0.188971000	4.364360000	-1.583394000
1	1.459095000	2.224204000	-1.164914000
1	0.910839000	2.959435000	0.332662000
1	0.485210000	-3.143855000	-0.368313000
1	1.235146000	-2.334032000	0.999633000
1	-0.281403000	-4.192713000	1.764544000
1	-0.853980000	-2.629275000	2.342308000
1	-3.562154000	2.022636000	1.663134000
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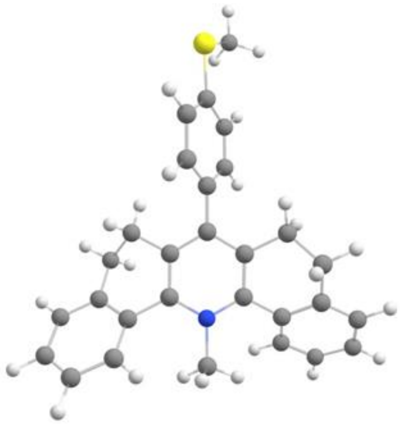

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1	-5.004728000	-4.092115000	-1.650951000
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6	-3.777485000	0.206362000	-0.381211000
1	-3.981237000	-0.016626000	-1.425169000
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1	-4.115469000	1.211115000	-0.184978000
7	-2.311821000	0.112802000	-0.149901000
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6	2.735201000	0.487074000	0.821345000
6	2.591009000	-0.877813000	-1.153964000
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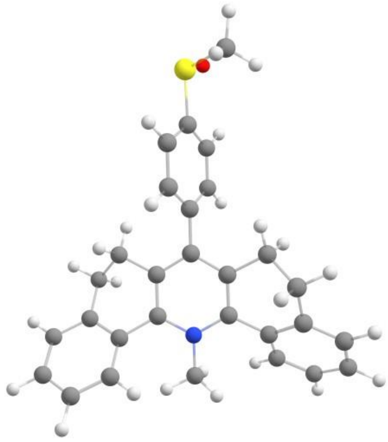
6	3.975154000	-0.969123000	-1.194909000
1	2.005360000	-1.371999000	-1.920797000
6	4.760292000	-0.339806000	-0.215762000
1	4.695973000	0.879749000	1.571891000
1	4.449191000	-1.533706000	-1.990173000
16	6.523792000	-0.530009000	-0.358939000
6	7.185993000	0.393569000	1.065675000
1	6.844588000	-0.033872000	2.008137000
1	8.268199000	0.284661000	0.996383000
1	6.930047000	1.451123000	1.006408000
1	-3.474046000	6.203423000	0.752332000
1	-4.257724000	-5.864071000	-0.069677000

S39 Ground State PM-SO

6	0.399584000	1.145862000	0.112219000

6	1.783487000	1.257846000	-0.094443000
6	1.953765000	-1.130452000	-0.113618000
6	0.563387000	-1.242917000	-0.220288000
6	-0.224209000	-0.096749000	-0.055969000
6	2.725726000	-2.373633000	0.120491000
6	2.255964000	-3.549954000	-0.499281000
6	0.981332000	-3.461058000	-1.295939000
6	-0.019348000	-2.609719000	-0.502278000
6	-0.362092000	2.382471000	0.526374000
6	0.532278000	3.247590000	1.420750000
6	1.761341000	3.614692000	0.632923000
6	2.360543000	2.626548000	-0.184384000
6	3.777416000	-2.459676000	1.048677000
6	4.417331000	-3.672156000	1.278940000
6	4.007983000	-4.817281000	0.595923000
6	2.920112000	-4.754786000	-0.272261000
6	2.257620000	4.916872000	0.618210000
6	3.301381000	5.277363000	-0.230347000
6	3.833119000	4.331718000	-1.103540000
6	3.366634000	3.021526000	-1.082947000
1	1.161379000	-2.997426000	-2.273057000
1	0.574548000	-4.456582000	-1.478028000
1	-0.949571000	-2.489908000	-1.059032000
1	-0.273324000	-3.118588000	0.435042000
1	-0.672998000	2.959115000	-0.352948000
1	-1.269942000	2.088023000	1.052557000
1	0.003836000	4.147557000	1.737904000
1	0.796179000	2.687560000	2.325952000
1	4.081436000	-1.590112000	1.617977000
1	5.225966000	-3.722606000	1.998114000
1	2.564310000	-5.654976000	-0.761143000
1	1.799882000	5.660900000	1.260734000

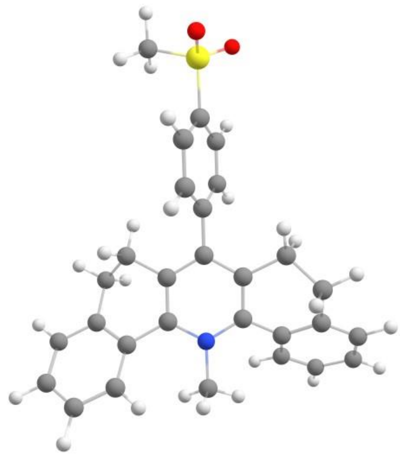


1	4.607458000	4.609378000	-1.808497000
1	3.775518000	2.329165000	-1.801093000
6	3.997416000	0.209028000	-0.447645000
1	4.141879000	0.437293000	-1.500272000
1	4.437864000	0.981868000	0.175329000
1	4.470810000	-0.738183000	-0.245944000
7	2.538537000	0.112129000	-0.175967000
6	-1.714075000	-0.200661000	-0.032620000
6	-2.364184000	-0.840141000	1.030808000
6	-2.473023000	0.334317000	-1.079285000
6	-3.752308000	-0.937529000	1.053144000
1	-1.787033000	-1.259944000	1.846185000
6	-3.862746000	0.233729000	-1.067567000
1	-1.979377000	0.820764000	-1.912655000
6	-4.488951000	-0.387835000	0.005637000
1	-4.243154000	-1.441642000	1.878273000
1	-4.459361000	0.628606000	-1.881091000
16	-6.307228000	-0.554940000	-0.008351000
6	-6.683107000	0.761313000	1.206575000
1	-6.285264000	1.706365000	0.837518000
1	-7.768707000	0.800758000	1.292676000
1	-6.240014000	0.490810000	2.165128000
1	4.511867000	-5.761700000	0.765672000
1	3.672145000	6.295774000	-0.233965000
8	-6.783088000	-0.034509000	-1.365690000

S40 Ground State PM-SO_2

6	-0.687680000	-1.167786000	0.108272000
6	-2.077292000	-1.207063000	-0.086996000
6	-2.121230000	1.187121000	-0.117918000
6	-0.727657000	1.225213000	-0.237845000
6	-0.002812000	0.039358000	-0.073009000

6	-2.823226000	2.470284000	0.118613000
6	-2.295795000	3.618237000	-0.508118000
6	-1.035196000	3.459568000	-1.316214000
6	-0.074163000	2.557314000	-0.529893000
6	0.011955000	-2.440625000	0.521703000
6	-0.919767000	-3.254568000	1.426132000
6	-2.172636000	-3.559019000	0.649046000
6	-2.725639000	-2.543559000	-0.167409000
6	-3.861184000	2.614862000	1.055077000
6	-4.432362000	3.860637000	1.287771000
6	-3.967247000	4.980592000	0.598726000
6	-2.891842000	4.857638000	-0.278408000
6	-2.736955000	-4.833222000	0.643014000
6	-3.804810000	-5.140929000	-0.196292000
6	-4.292409000	-4.171789000	-1.069554000
6	-3.757512000	-2.887981000	-1.057559000
1	-1.248814000	3.004358000	-2.290501000
1	-0.577209000	4.431418000	-1.504269000
1	0.842401000	2.386733000	-1.096149000
1	0.216198000	3.053480000	0.403740000
1	0.285624000	-3.035516000	-0.357854000
1	0.937793000	-2.192949000	1.040766000
1	-0.436743000	-4.179994000	1.742306000
1	-1.146538000	-2.678523000	2.331310000
1	-4.207374000	1.764281000	1.628907000
1	-5.230999000	3.956164000	2.013471000
1	-2.492079000	5.736232000	-0.772493000
1	-2.314270000	-5.598221000	1.284907000
1	-5.085312000	-4.410876000	-1.767975000
1	-4.133763000	-2.177849000	-1.776247000
6	-4.235781000	-0.044652000	-0.425479000
1	-4.402987000	-0.271967000	-1.474834000



1	-4.709949000	-0.789124000	0.207359000
1	-4.656143000	0.927686000	-0.225921000
7	-2.770933000	-0.023278000	-0.168807000
6	1.491387000	0.064327000	-0.064467000
6	2.183386000	0.641705000	1.006647000
6	2.206790000	-0.488260000	-1.133130000
6	3.574622000	0.662017000	1.017618000
1	1.637449000	1.071089000	1.837974000
6	3.598321000	-0.466085000	-1.135959000
1	1.678272000	-0.926964000	-1.970944000
6	4.266149000	0.107114000	-0.056647000
1	4.110023000	1.112433000	1.843622000
1	4.153026000	-0.879144000	-1.968609000
16	6.069103000	0.126392000	-0.046520000
6	6.550675000	-1.382194000	0.811592000
1	6.162875000	-2.235219000	0.257633000
1	7.640754000	-1.387466000	0.821994000
1	6.152522000	-1.348373000	1.824276000
1	-4.417711000	5.951263000	0.770504000
1	-4.228822000	-6.138338000	-0.193107000
8	6.532572000	0.028997000	-1.443505000
8	6.507695000	1.276821000	0.766034000

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