Supporting Information

Near-IR Nanolignin Sensitizers Based on Pyrene Conjugated Chlorin and Bacteriochlorin for ROS Generation, DNA Intercalation and Bioimaging

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S1. Chemicals and instruments

Chemicals. The chemicals/reagents/solvents were purchased from CDH Chemicals, SRL Chemicals, TCI Chemicals (India) Pvt. Ltd., and Sigma-Aldrich; Merck Chemicals, Germany. These chemicals and reagents were used without purification. The analytical grade solvents were used in the column chromatography. The characterization of *meso*-phenyl dipyrromethane (**5**) and 1,9-Diformyl-5-(4-phenyl)dipyrromethane (**6**) was consistent with previously reported data.[1] The column chromatography of PyChl (**9a**) and PyBac (**9b**) was performed on silica gel (100-200 mesh). Solvent system: Petroleum ether and dichloromethane (DCM) [ratio 3:1].

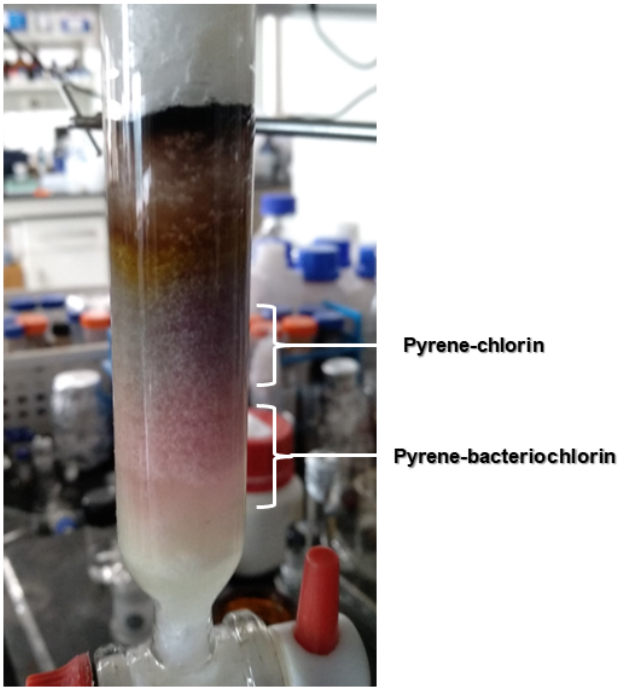


Figure S1. Digital photograph of column chromatography of the photosensitizers. The first pink band was identified as pyrene-bacteriochlorin (**9b**), and the second green band was identified as pyrene-chlorin (**9a**).

Instruments and equipment. The microwave-assisted reactions were performed using the microwave synthesizer (CEM Discover system; Model no. 908010). ^{1}H and ^{13}C NMR spectra were recorded on a Brucker Advance Neo 500 MHz NMR spectrometer at SAIF, Panjab University, Chandigarh. ^{1}H NMR was analyzed using DMSO-d_6 or $CDCl_3$ as solvents. The UV−Vis spectra were recorded on a Shimadzu UV-2600. The fluorescence emission spectra were recorded using a Cary Eclipse Fluorescence Spectrophotometer (Agilent) at CIF, CIAB Mohali. The mass spectra were recorded on an LC−MS Spectrometer Q-TOF Micro at SAIF, Panjab University, Chandigarh. The UPLC data were performed by the Waters Acquity UPLC system at CIF, CIAB Mohali. The solvents

(HPLC grade) were MillQ water (0.1% TFA, solvent A) and acetonitrile (0.1% TFA, solvent B). 0−20 min; isocratic solvent system (1:9), respectively. HPLC data were collected using Waters 1525 (Empower software) system (same solvent system as UPLC was used, except for PyBac in which no TFA was added). The mean particle size and zeta potential were determined by a zetasizer (Malvern; Nano 25) at CIF, CIAB Mohali. Transmission electron microscopy (FEI, USA) was performed at CIL, NIPER Mohali.

S2. Reaction modification/optimization

Table S1. Optimization of diimide reduction conditions (using microwave synthesizer)

Entry	Base (100 eq.)	Temperature (°C)	Time (min)	Yield (%)	
				PyChl (9a)	PyBac (9b)
1	K_2CO_3	120	25	18 – 19	19 – 20
2	K_2CO_3	130	25	19	22
3	K_2CO_3	120	30	20	18
4	K_2CO_3	130	30	20	20
5	K_2CO_3 (25 eq in every 15 min)	120	60	9	10
6	–[a]	120	30	15	15
7	–[a]	130	30	18	19

[a] The reactions were not reproducible

S3. Characterization data

S3.1 ^{1}H and ^{13}C-NMR spectra.

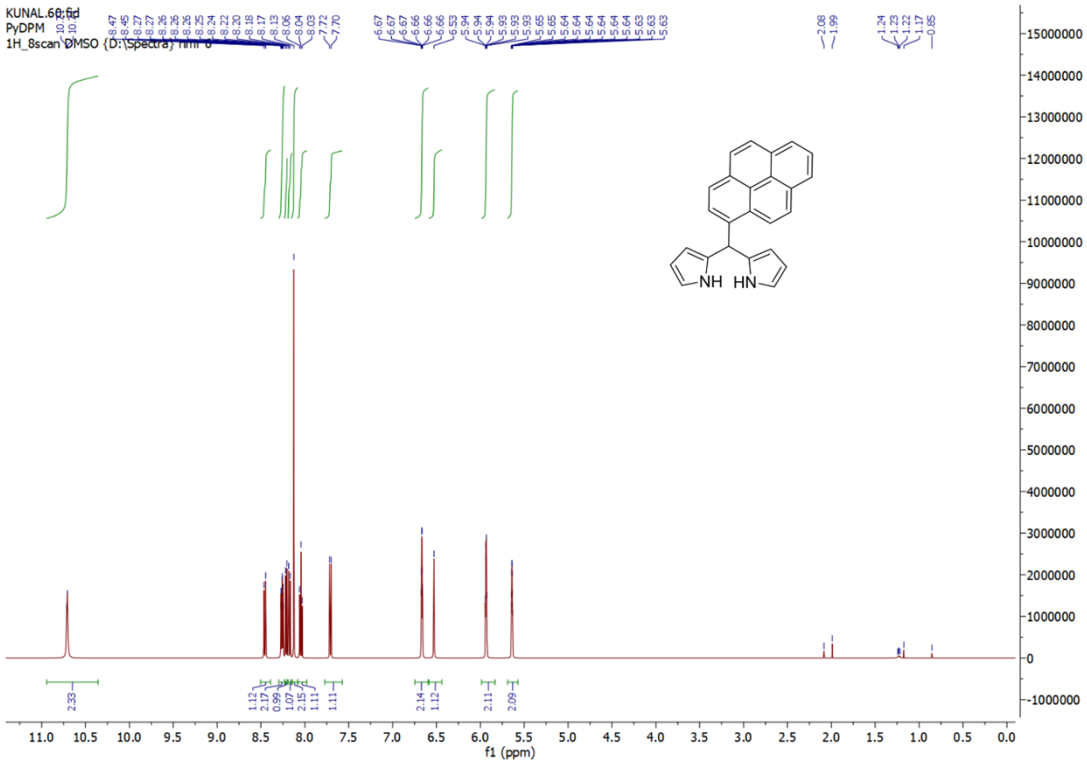


Figure S2. ^{1}H-NMR spectrum of meso-pyrene dipyrromethane (**3**).

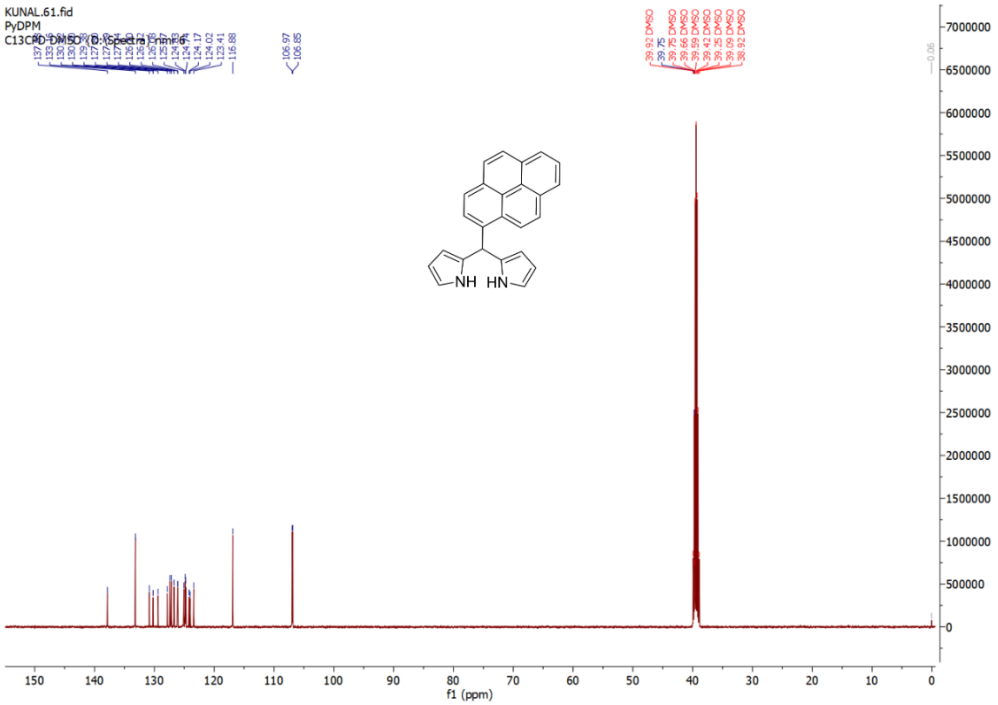


Figure S3. ^{13}C-NMR spectrum of meso-pyrene dipyrromethane (**3**).

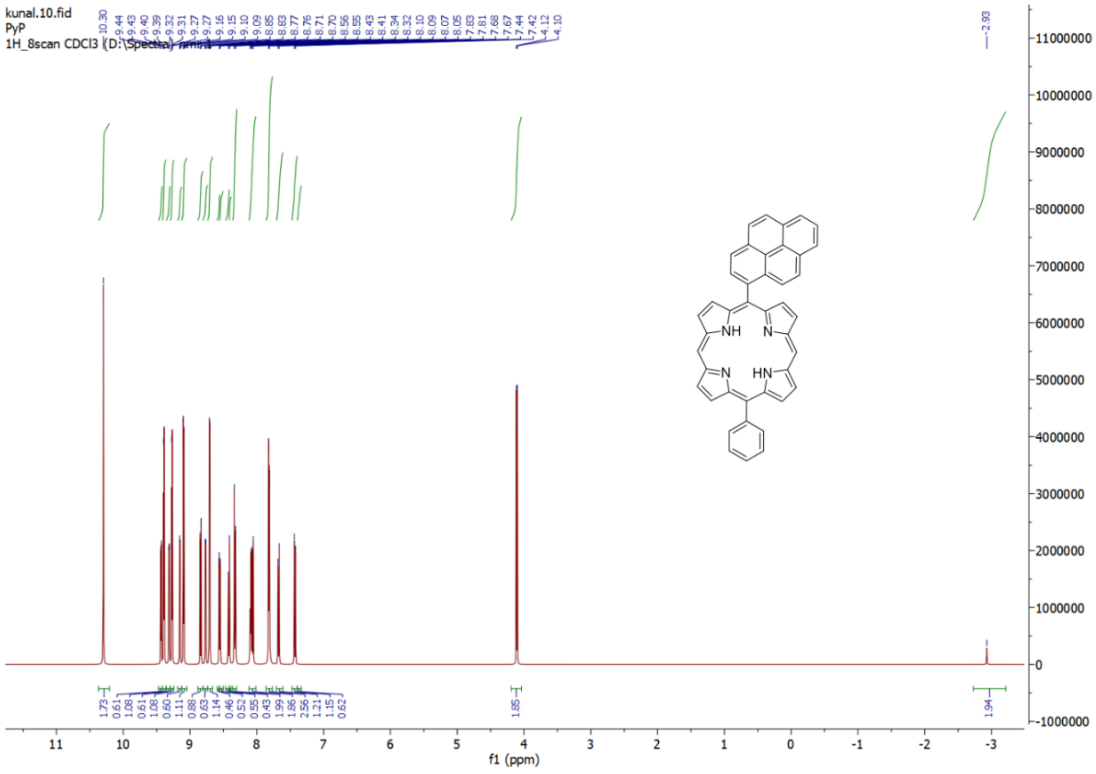


Figure S4. ^{1}H-NMR spectrum of pyrene-porphyrin (**8**).

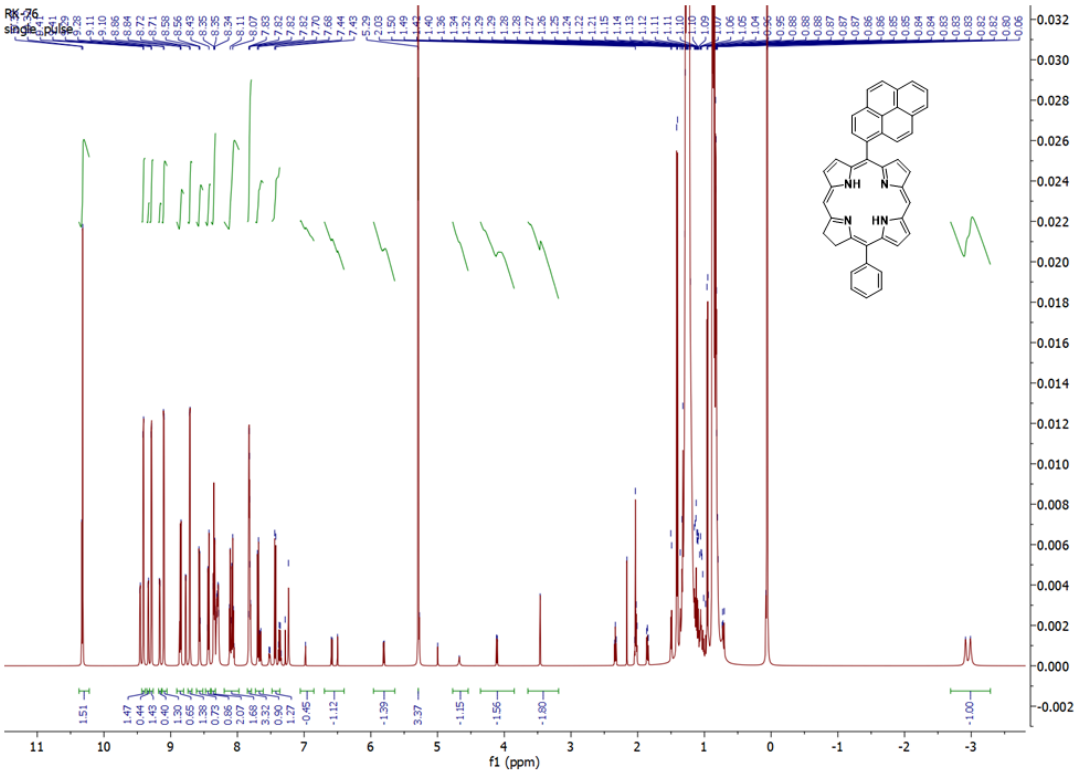


Figure S5. ^{1}H-NMR spectrum of pyrene-chlorin (**9a**).

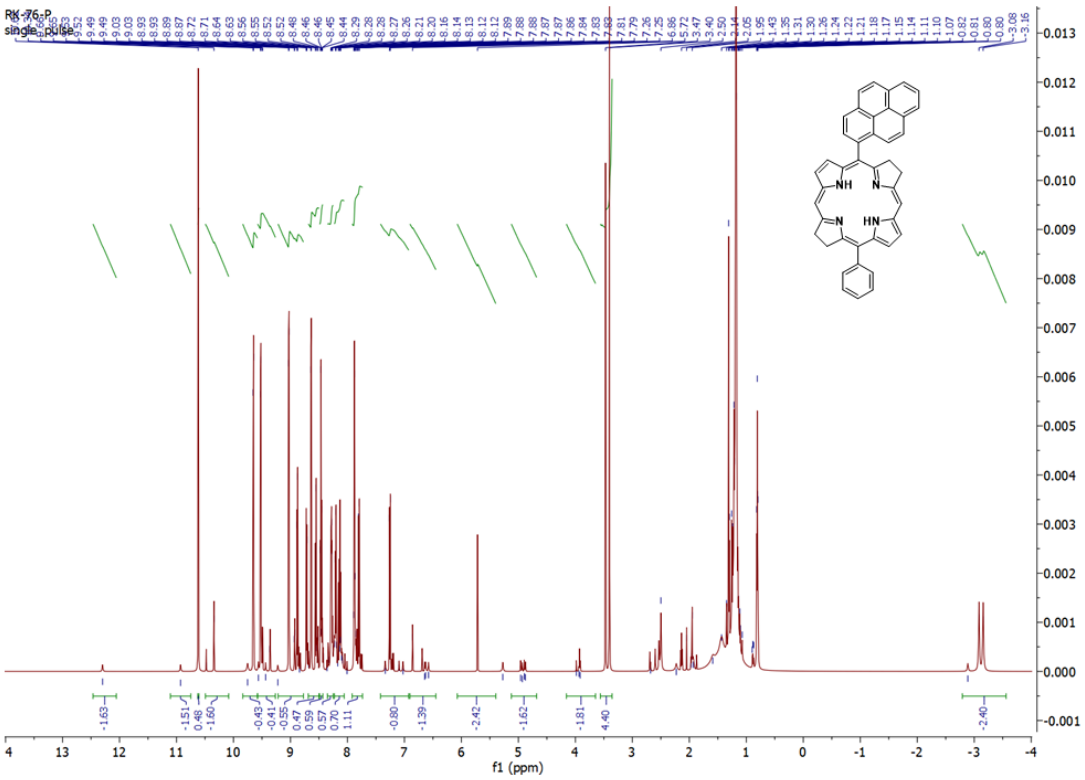


Figure S6. ^{1}H-NMR spectrum of pyrene-bacteriochlorin (**9b**).

S3.2 Mass spectra

PyDPM (**3**): HPLC-MS: ($C_{25}H_{18}N_2$) m/z $[M]^+$ calc. 346.15; found 346.15.

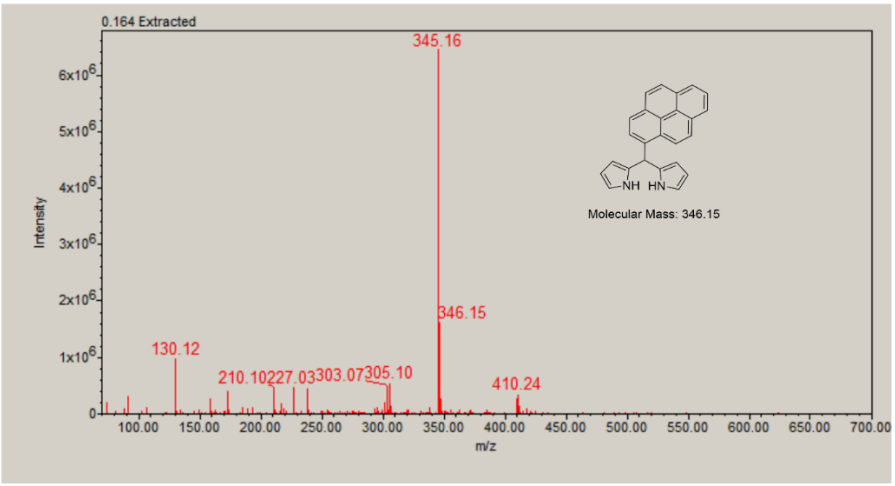
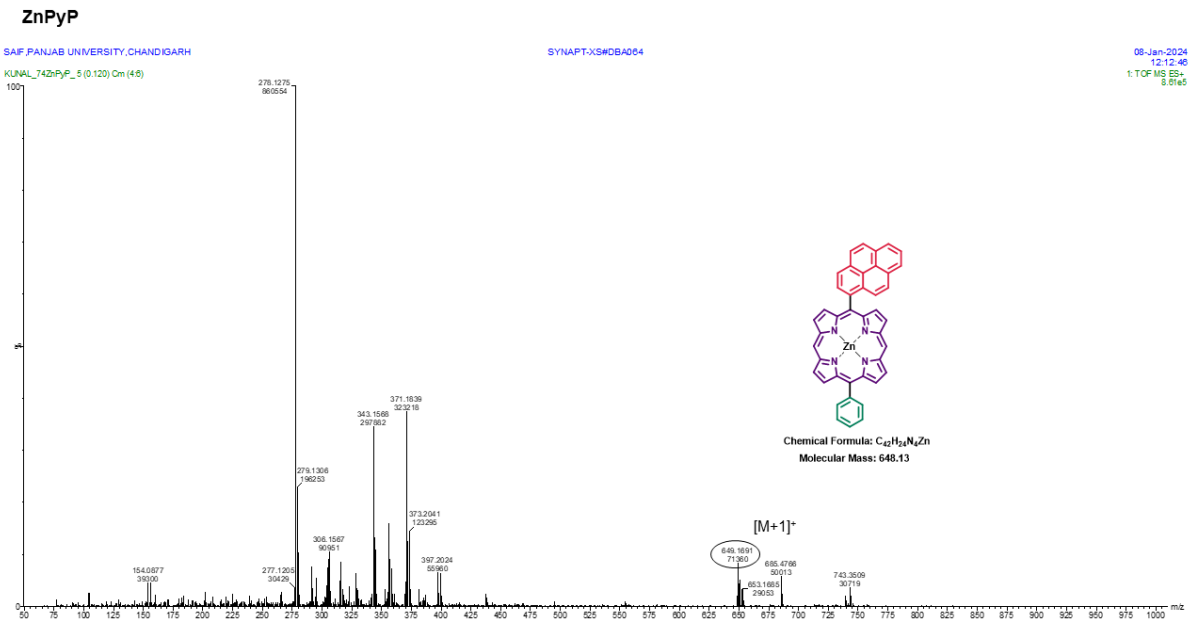


Figure S7. HPLC-MS spectrum of meso-pyrene dipyrromethane (**3**).



Figur e S8. ESI-MS spectrum of ZnPyP (**7**).

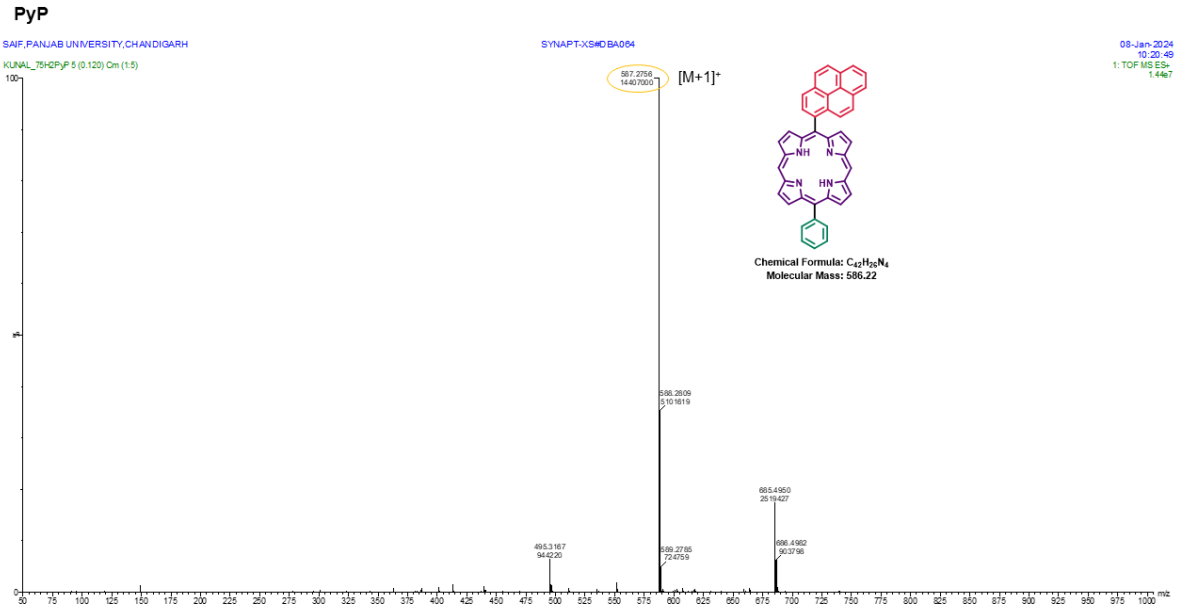


Figure S9. ESI-MS spectrum of PyP (**8**).

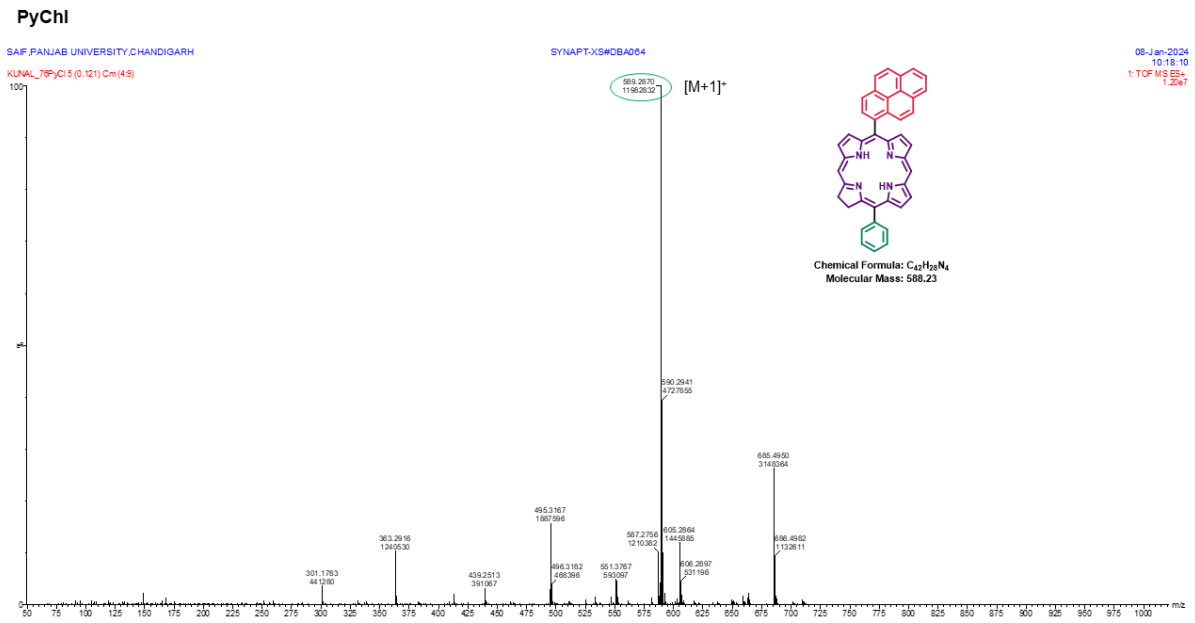


Figure S10. ESI-MS spectrum of PyChl (**9a**).

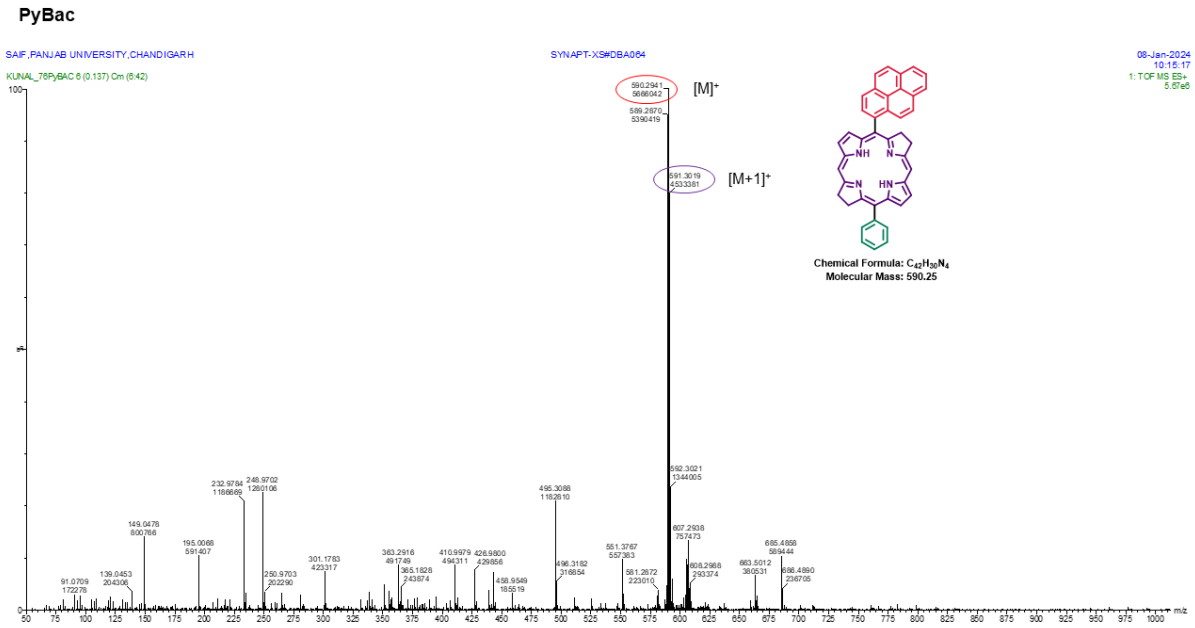


Figure S11. ESI-MS spectrum of PyBac (**9b**).

S3.3 High performance liquid chromatography

High-performance liquid chromatography was performed to check the purity of pyrene-conjugated Zn-metalated porphyrin, free base porphyrin, chlorin and bacteriochlorin (**7**, **8**, **9a**, and **9b**). The HPLC system was Waters HPLC system from (USA), regulated by the EmpowerTM pro software. The autosampler and analytical C18 column (5 µm, 4.6 × 250 mm Column) was employed for this

study. The detection was executed using the Waters eλ PDA detector at a temperature of 30°C and a wavelength range of 200-800 nm. HPLC analysis was performed isocratically in 0.1% TFA in the acetonitrile and water composition (9:1) run for ZnPyP, PyP, and PyChl. Only acetonitrile and water composition (9.5:5) was used for PyBac. The flow rate for all the samples was 1 mL/min.

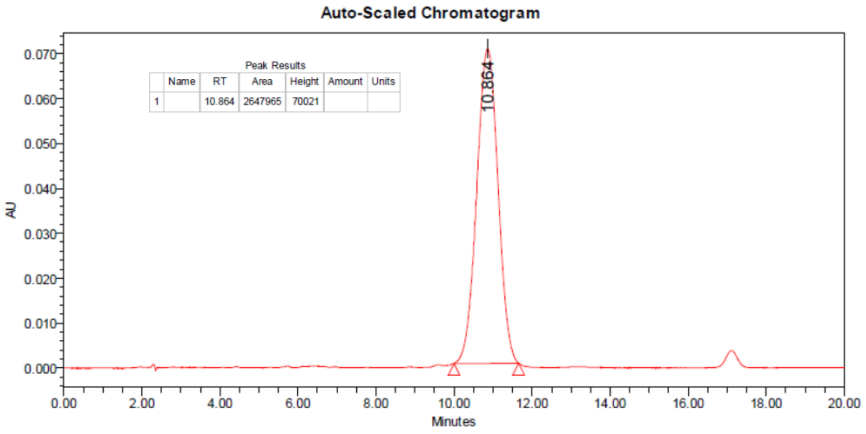


Figure S12. HPLC data of ZnPyP (**7**). t_R = 10.86 [the analysis was performed isocratically in 0.1% TFA in the acetonitrile and water composition (90:10) run over 20 min, purity >99%].

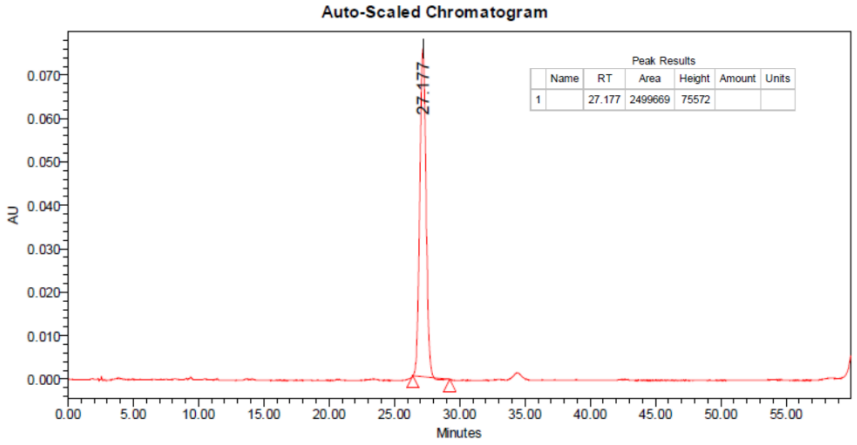


Figure S13. HPLC data of PyP (**8**). t_R = 27.18 [the analysis was performed isocratically in 0.1% TFA in the acetonitrile and water composition (90:10) run over 60 min, purity >99%].

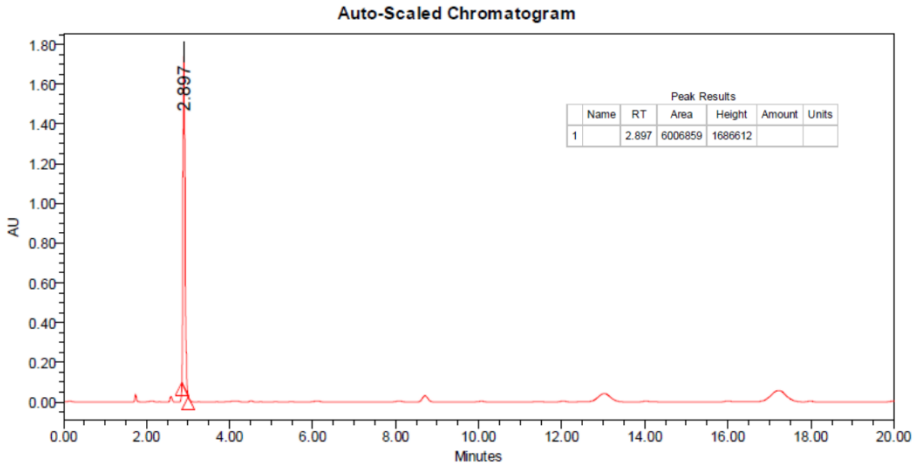


Figure S14. HPLC data of PyChl (**9a**). t_R = 2.89 [the analysis was performed isocratically in 0.1% TFA in the acetonitrile and water composition (90:10) run over 20 min, purity >99%].

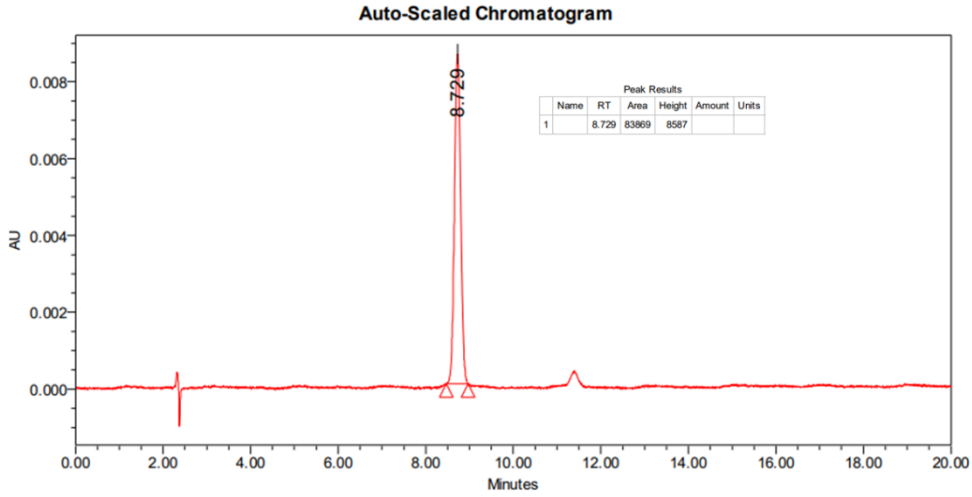


Figure S15. HPLC data of PyBac (**9b**). t_R = 8.72 [the analysis was performed isocratically in the acetonitrile and water composition (95:5) run over 20 min, purity >98%].

S3.4 UV-Vis spectra

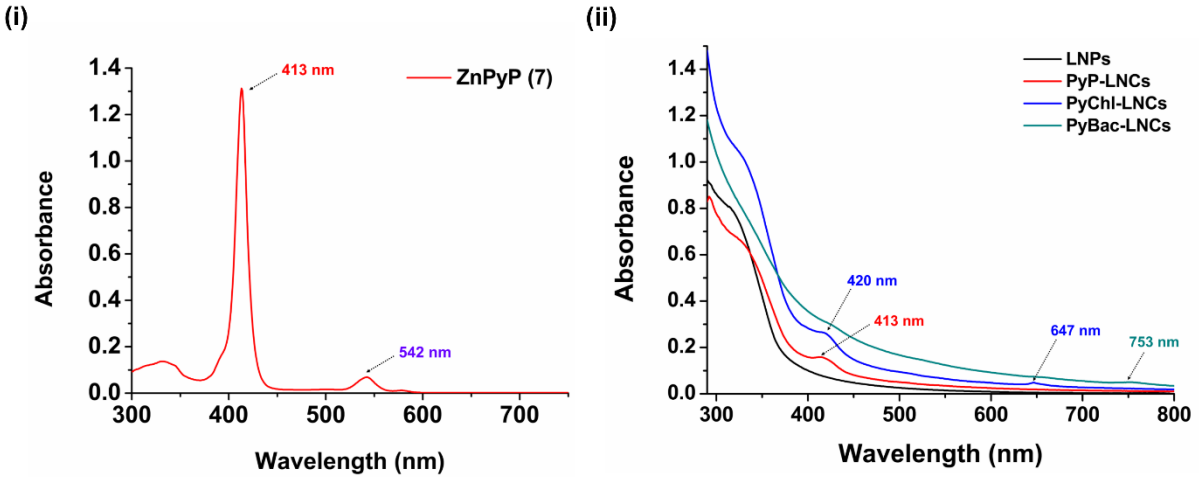


Figure S16. (i) UV-Vis spectra of ZnPyP (**7**), and (ii) UV-Vis spectra of LNPs, PyP-LNCs, PyChl-LNCs, and PyBac-LNCs.

S3.5 Mean particle size and zeta potential

Table S2. Characterization data of pyrene-porphyrin-loaded lignin nanoparticles (PyP-LNCs), pyrene-chlorin-loaded lignin nanoparticles (PyChl-LNCs) and pyrene-bacteriochlorin-loaded lignin nanoparticles

Nanoparticles	Mean particle size (Z-average in nm)	Polydispersity index (PDI)	Zeta potential (mV)
LNPs	74 ± 2	0.171 ± 0.004	-30 ± 1
PyP-LNCs	112 ± 4	0.233 ± 0.005	-31 ± 1
PyChl-LNCs	126 ± 3	0.055 ± 0.01	-29 ± 2
PyBac-LNCs	115 ± 6	0.096 ± 0.005	-34 ± 3

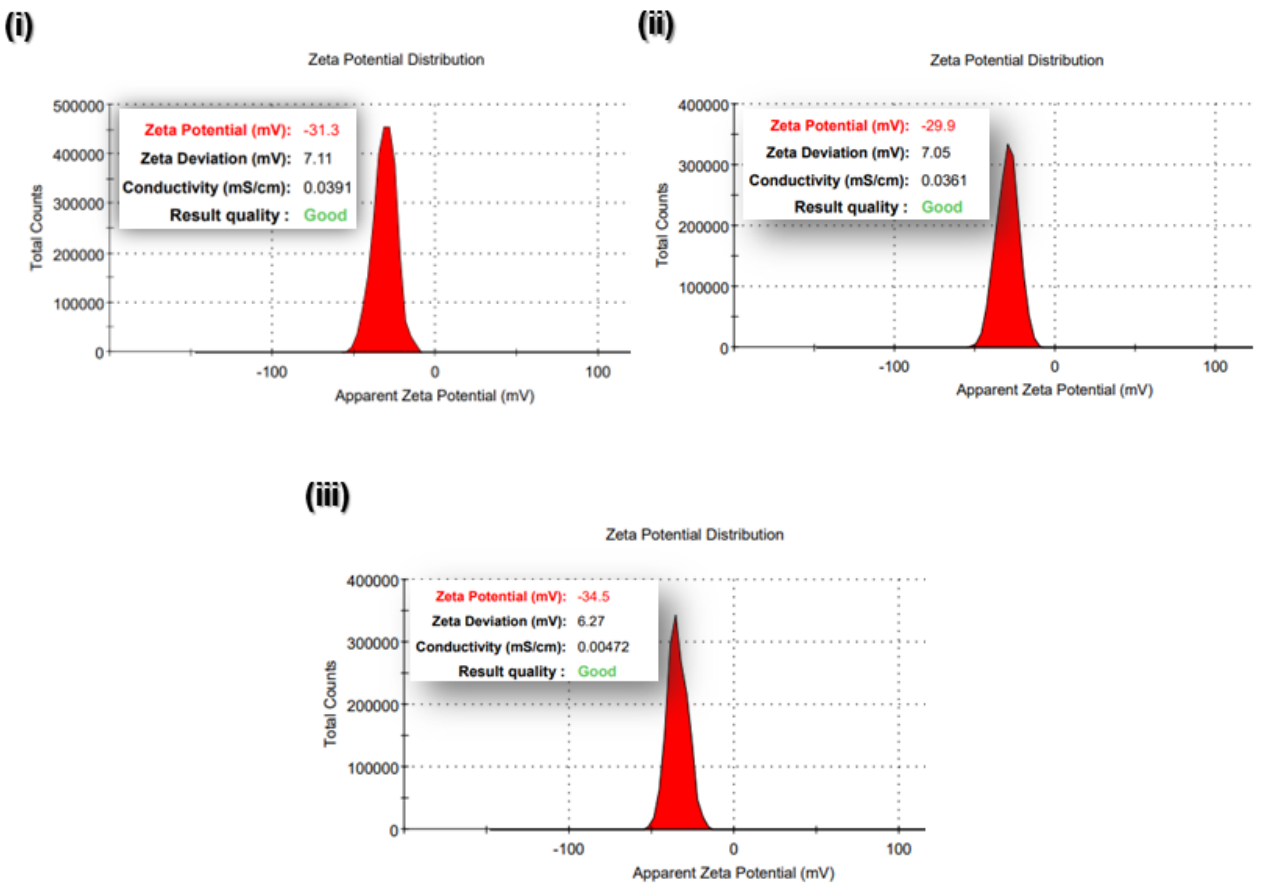


Figure S17. Zeta potential of nano-formulations: (i) PyP-LNCs, (ii) PyChl-LNCs, (iii) PyBac-LNCs.

S3.6 Loading efficiency (%)

The standard calibration curves of the photosensitizer were prepared, and linear regression equations were obtained. The supernatant containing photosensitizer was determined by UV-Vis spectroscopy.

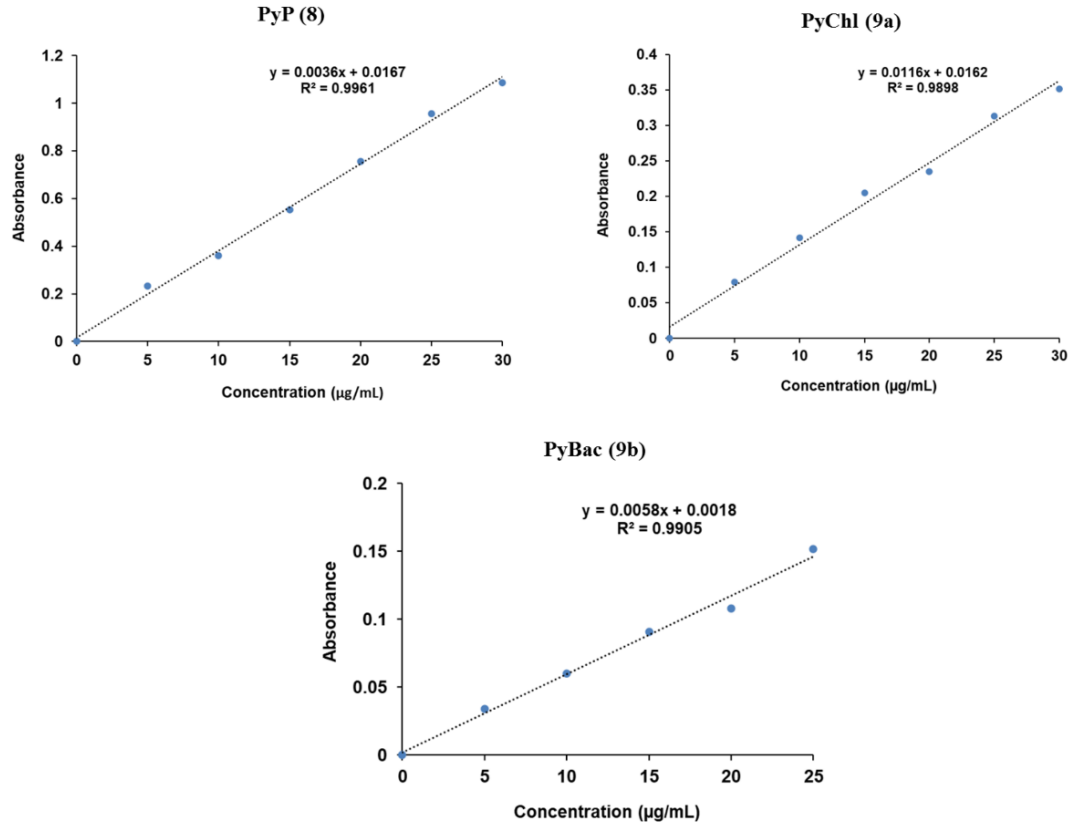


Figure S18. Standard calibration curves of PyP, PyChl, and PyBac recorded by UV-Vis spectroscopy.

Calculation:

The loading efficiency (%) of each nanoformulations (PyP-LNCs, PyChl-LNCs and PyBac-LNCs) were assessed by the following equation (1)

Loading efficiency % = [(Total drug added - free drug in supernatant)/Total drug added] × 100 (1)

Table S3. % Loading analysis of different photosensitizers (**8**, **9a**, **9b**) in lignin nanoparticles

Nanoparticles	**% Loading**			**Mean**	**SD**
	S1	**S2**	**S3**	**(%)**	
PyP-LNCs	95	94	93	94	1
PyChl-LNCs	90	90	91	90.33	0.47
PyBac-LNCs	91	89	90	90	0.81

S3.7 Stability studies

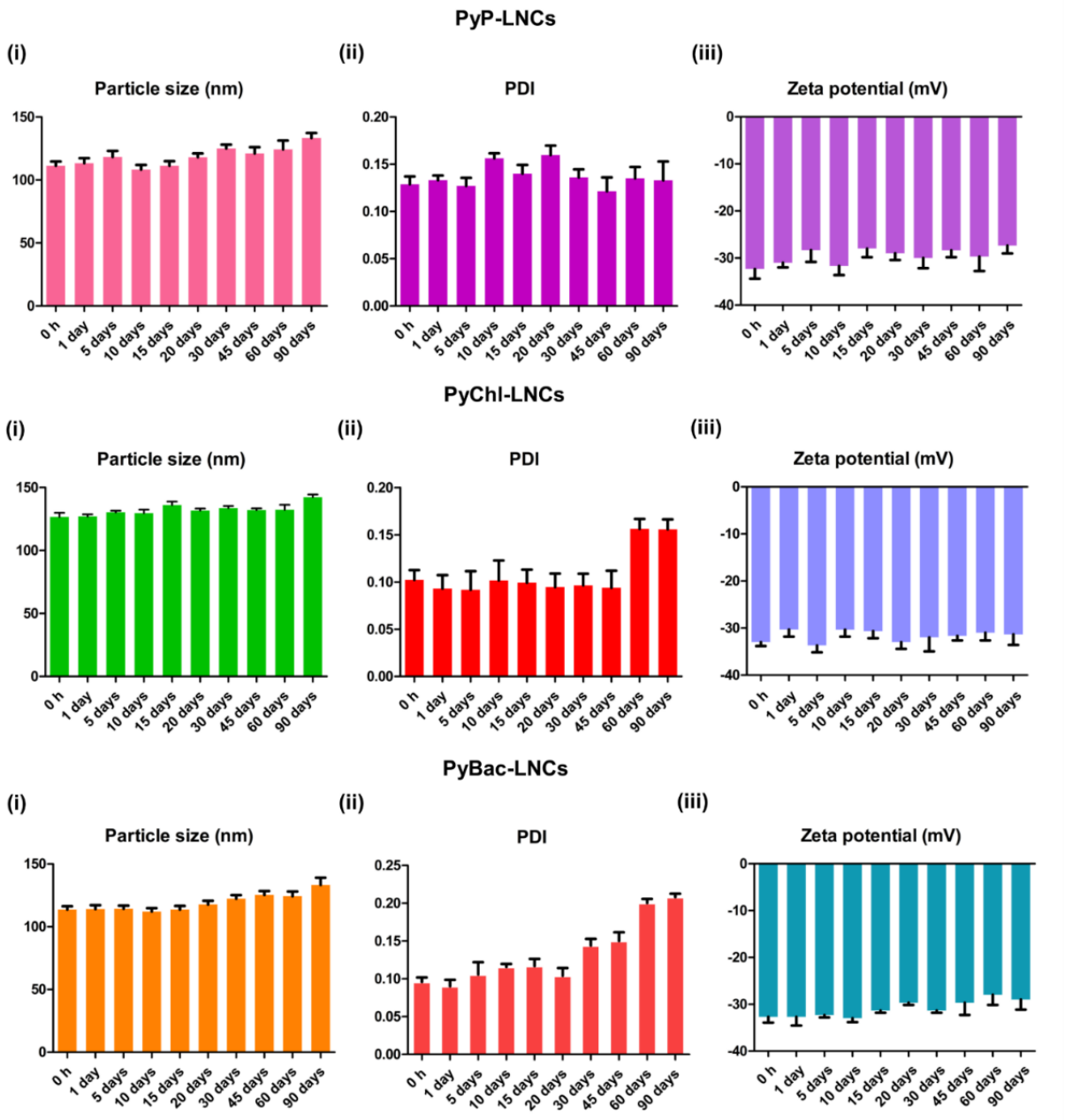


Figure S19. Stability study of the nanoformulations (PyP-LNCs, PyChl-LNCs, and PyBac-LNCs) in terms of (i) mean particle size, (ii) polydispersity index (PDI), and (iii) zeta potential up to 90 days.

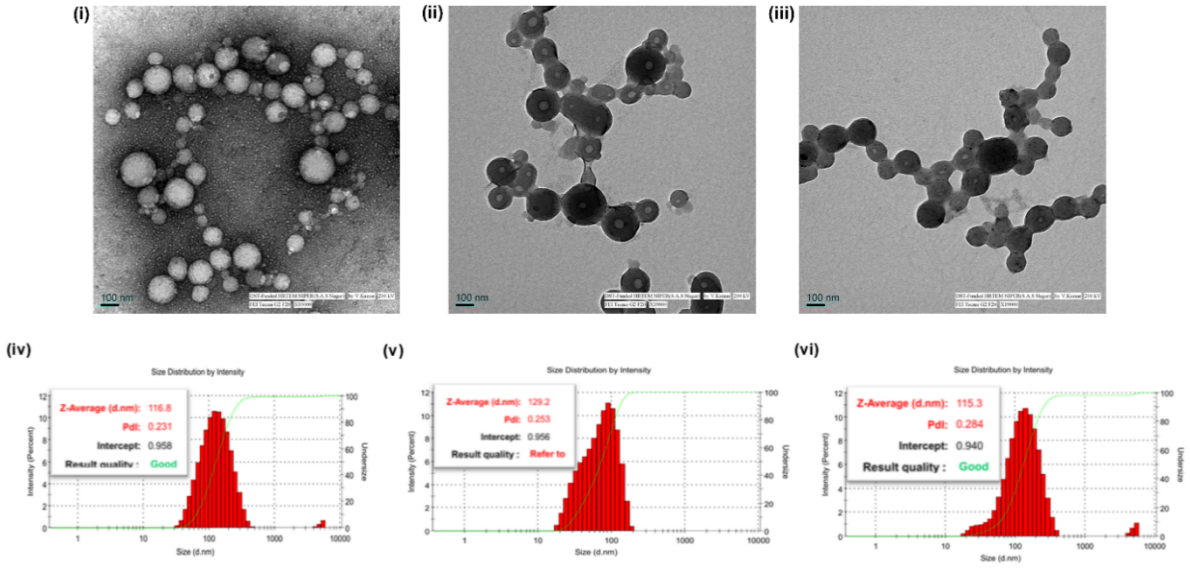


Figure S20. Stability studies of the nanoformulations (i) PyP-LNCs, (ii) PyChl-LNCs, and (iii) PyBac-LNCs; morphology of nanoparticles, the morphology was observed using transmission electron microscopy (TEM) and DLS data/mean particle size of nano-formulations: (iv) PyP-LNCs, (ii) PyChl-LNCs, and (vi) PyBac-LNCs after 1 month storage at 4 °C

S4. Singlet oxygen quantification graphs

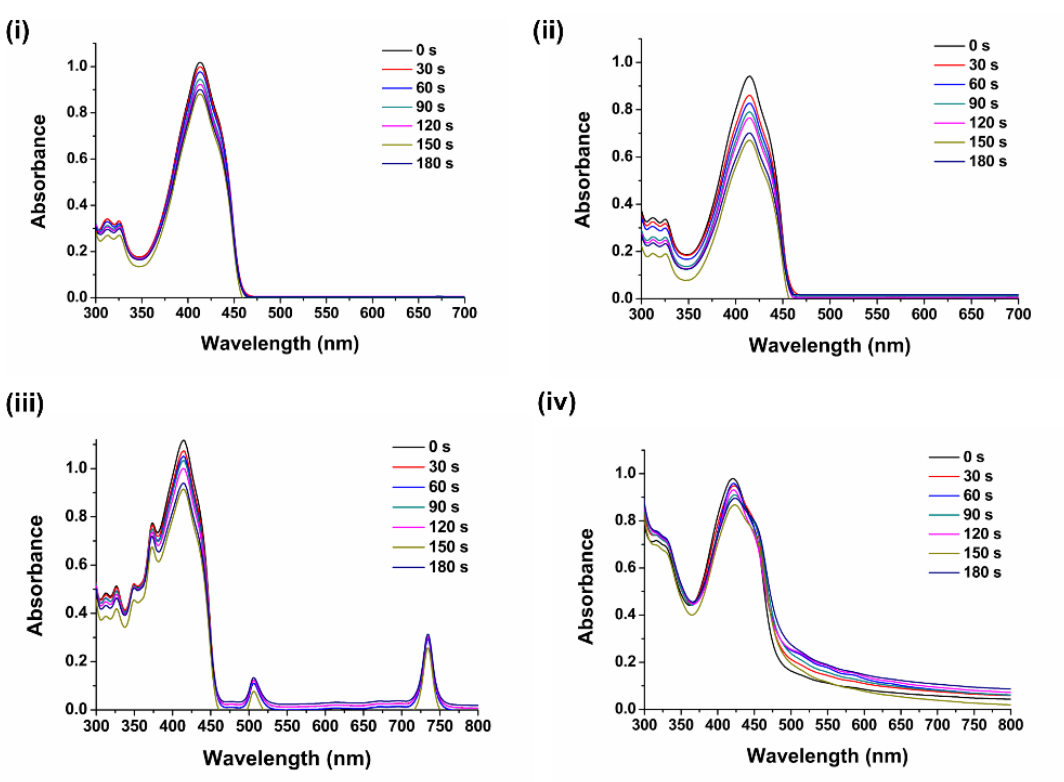


Figure S21. Singlet oxygen-mediated degradation of DPBF upon 30 s time interval exposure to the green LED (12 W; 500-570 nm, 32,400 J/cm^2)/red LED (12 W; 620-750 nm, 32,400 J/cm^2): (i) graph

represents the DPBF absorbance upon 30 s exposure with PyP (**8**) porphyrin under green LED, (ii) reduction in DPBF absorbance after exposure with PyChl (**9a**) under red LED, (iii) absorbance of DPBF solutions after exposure with PyBac (**9b**) under green LED, and (iv) absorbance of DPBF after exposure with PyP-LNCs under red LED.

S5. Photoluminescence studies

Table S4. Fluorescence quantum yield calculations of **8**, **9a**, and **9b** by standard relative method

Photosensitizer	Integrated intensity (n=3)	Absorbance at excitation wavelength (n=3)	Fluorescence quantum yield (ϕ_F)
PyP (8)	7111 ± 7	0.158 ± 0.01	0.55 ± 0.07
PyChl (9a)	7108 ± 5	0.175 ± 0.02	0.85 ± 0.12
PyBac (9b)	7669 ± 11	0.113 ± 0.01	0.66 ± 0.03
ZnPc (ref)	8279	0.37	-
(**8**): λ_{ex} = 418 nm, (**9a**): λ_{ex} = 406 nm, and (**9b**): λ_{ex} = 507 nm			

S6. Morphological observation of *Candida albicans* before and after treatment

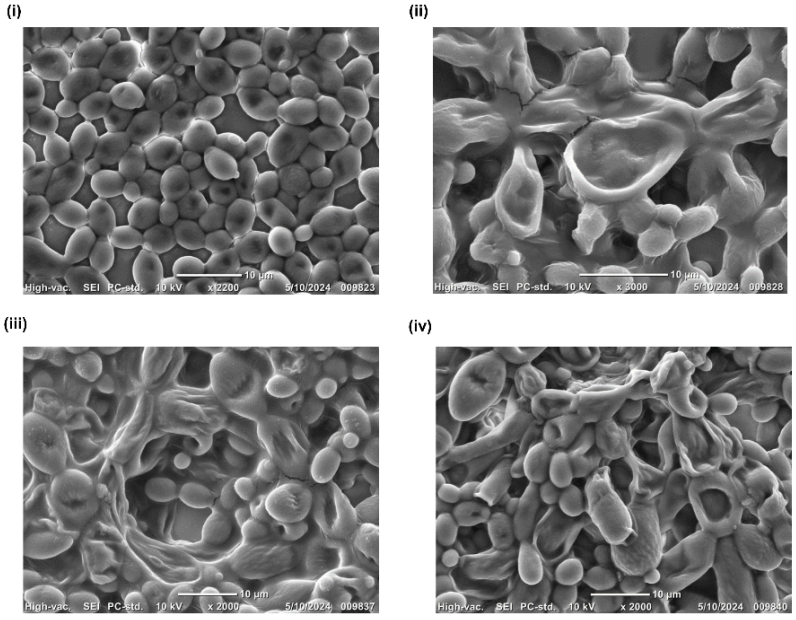


Figure S22. (i) Morphology of *C. albicans* cells without any treatment, (ii) structural changes in *C. albicans* cells after treatment with PyP-LNCs under green LED (12 W; 500-570 nm, 32,400 J/cm^2), (iii) *C. albicans* cells after treatment with PyChl-LNCs under red LED (12 W; 620-750 nm, 32,400 J/cm^2), and (iv) *C. albicans* cells after treatment with PyBac-LNCs under red LED (12 W).

S7. Intracellular ROS generation analysis

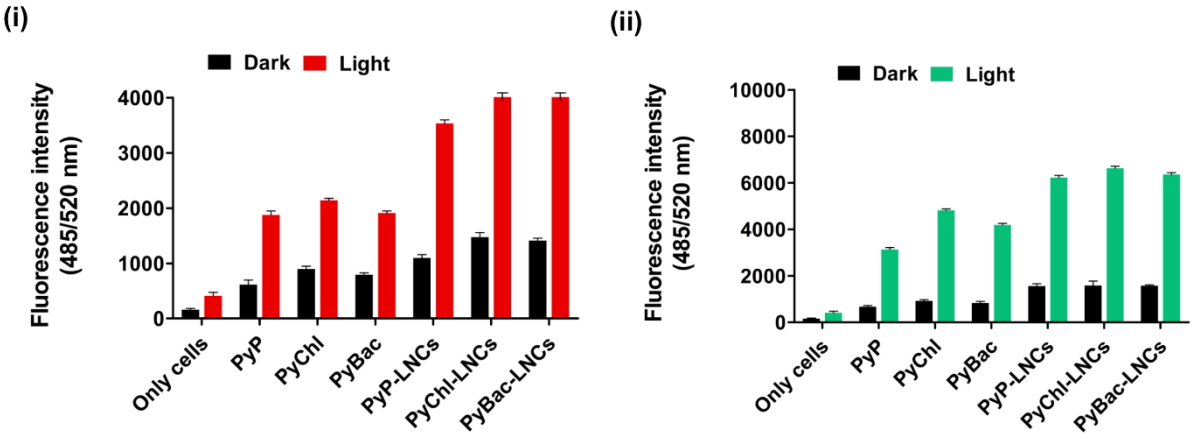


Figure S23. ROS generation analysis with nanolignin senstizers (PyP-LNCs, PyChl-LNCs, and PyBac-LNCs) under light and dark conditions: (i) 5 µg/mL, and (ii) 10 µg/mL.

References

1. S. Paul, N. S. Thakur, S. Chandna, Y. N. Reddy and J. Bhaumik, *J Mater Chem B*, 2021, **9**, 1592–1603.
2. Y. N. Reddy, N. Singh Thakur and J. Bhaumik, *ChemNanoMat*, 2020, **6**, 239–247.
3. K. Gogde, S. Paul, A. K. Pujari, A. K. Yadav and J. Bhaumik, *J Med Chem*, 2023, **66**, 13058–13071.