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SUPPORTING INFORMATION

Molecular-engineered highly photosensitive triarylphosphine oxide compounds for apoptosis imaging and selectively inducing apoptosis of tumor cells by photodynamic

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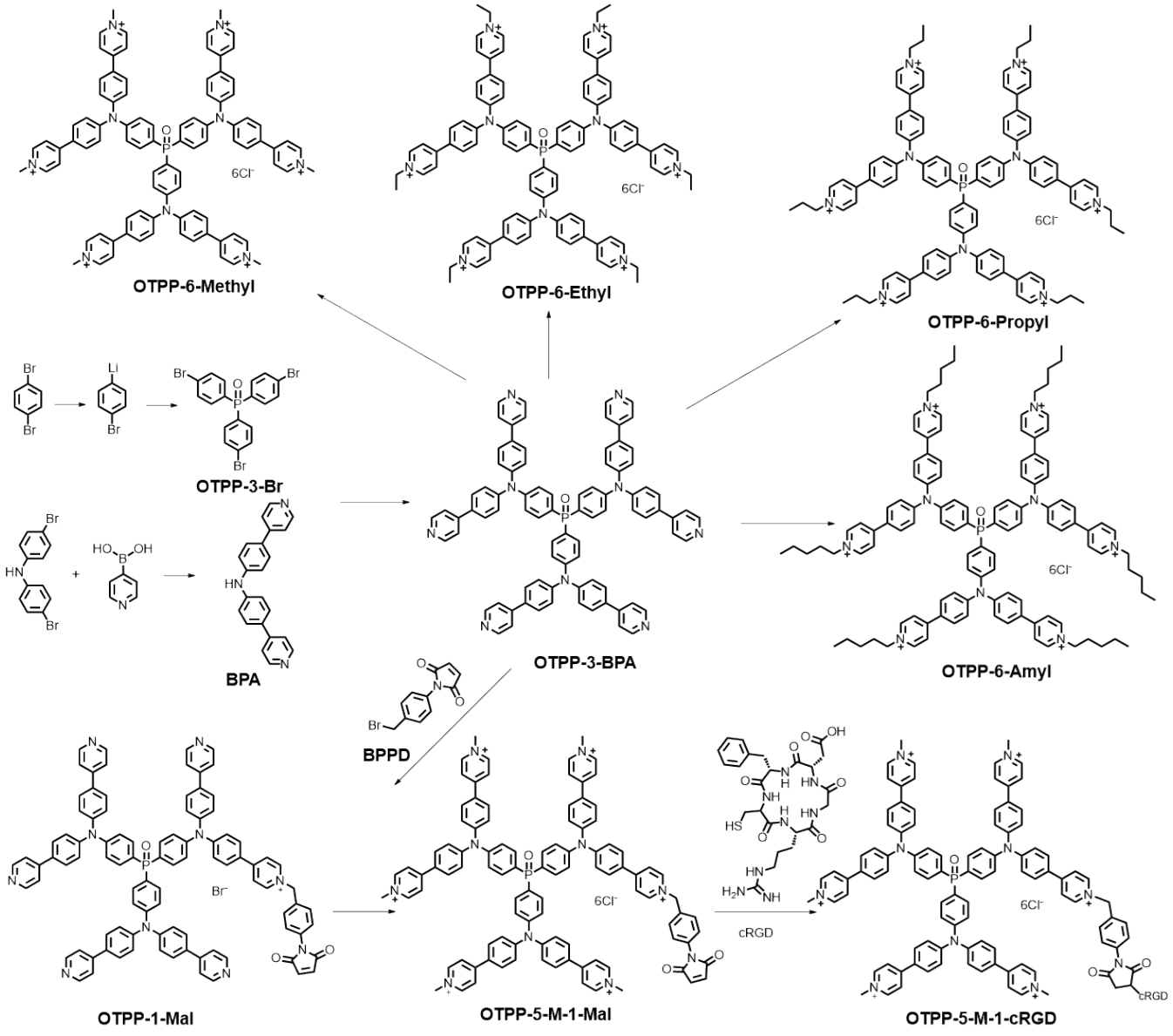
Experimental Procedures

1. General information

General chemical reagents were purchased from Beijing InnoChem Science &Technology Co (Beijing, China) and used without further purification. AV 647/PI apoptosis detection kit and JC10 was purchased form YEASEN biotechnology Co (Shanghai, China) and used according toits standard process. Absorption spectra were recorded on Hitachi UV-3010 (Hitachi, Tokyo, Japan). The fluorescence spectra were obtained on Hitachi F-7100 (Hitachi, Tokyo, Japan). Cells were analyzed using a confocalmicroscope (OLYMPUS FV 1000-IX81 Olympus Corporation, Tokyo, Japan). NMR spectra were obtained on BrukerAvance III 400 H (400 MHz) spectrometers (Bruker, Karlsruhe, Germany). In vivo small animal imaging system (In-Vivo MS FX PRO, Bruker, Germany).

2. Synthsis of OTPP-6-Methyl, OTPP-6-Ethyl, OTPP-6-Propyl, OTPP-6-Amyl and OTPP-5-M-1-cRGD.



Scheme S1. The synthetic route to compounds OTPP-6-Methyl, OTPP-6-Ethyl, OTPP-6-Propyl, OTPP-6-Amyl and OTPP-5-M-1-cRGD

OTPP-3-Br , cRGD, BPA and BPPD were synthesized according to the previous reported procedure. [1], [2], [3],[4]

Synthesis of Compound OTPP-3-BPA

To a mixture of compound OTPP-3-Br (0.512g, 1 mmol), BPA (1.454 g,4.5mmol), sodium tert-butoxide (0.864g,9mmol), tris(dibenzylideneacetone)dipalladium (36mg,0.04mmol), 2,2'-Bis(diphenylphosphino)-1,1'-binaphthy (0.12g,0.5 mmol), was added toluene (200 mL) and was refluxed at 120 °C for 3 days. Then the reaction mixture was cooled to room temperature, removed the solvent under vacuum. The residue was extracted with $CHCl_3$ then dried over sodium sulfate, filtered and evaporated under reduced pressure to get a yellow solid. The crude product was purified by column chromatography (EtOAc-Methol5:1) to get a yellow powder 0.705g yield: 58%.^{1}H NMR (400 MHz, CD_2Cl_2) δ: 7.25-7.27 (m, 6H), 7.31-7.33 (d, 12H, J=8),7.56-7.58 (m, 12H) ,7.62-7.64(d, 6H), 7.67-7.70(m, 12H), 8.65-8.67(m, 12H). ppm. ^{13}C NMR (100 MHz, CD_2Cl_2) δ:149.89,147.47, 133.47,133.34,133.23,128.20,125.49,122.39,122.26,121.10 ppm. MALDI-TOF (m/z): Calcd. For $C_{84}H_{60}N_9OP$ 1241.47, found 1242.47

Synthesis of Compound OTPP-6-Methyl

OTPP-3-BPA (62mg,0.05mmol), CH_3I 2mL was added to 25 mL bottom and stirred at room temperature, after stirring for 24 hours, then CH_3I was removed in vacuo. The residue was dissolved in water. KPF_6 saturated solution was added to the solution, filtered, the precipitate was dissolved in CH_3CN. The CH_3CN solution was added to a solution of excess tetrabutyl ammonium chloride in CH_3CN, filtered, the residue was dissolved in CH_3OH 200 uL excess ethylacetate was added to the solution, filtered, the precipitate was dryed in vacuum oven.The product compound *OTPP-6-Methyl* was obtained as a yellow solid (75mg, yield: 97%).^{1}H NMR (400 MHz, CD_3OD) δ: 4.41 (s, 18H), 7.42-7.44 (d, 18H,J=8), 7.77-7.82 (m, 6H), 8.09-8.11 (d, 12H,J=8), 8.40-8.41 (d, 12H,J=4) ,8.87-8.88(d,12H,J=4) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ:154.80,149.78,145.17,133.59,133.48,129.59,129.17,125.05,124.52,124.40,123.59 ppm.MALDI-TOF (m/z): Calcd. For $[C_{90}H_{78}N_9OP]^{6+}$ (M/Z,Z=5)266.32, found 266.52.

Synthesis of Compound OTPP-6-Ethyl

OTPP-3-BPA (62mg,0.05mmol), bromoethane (108.9mg,1mmol), N,N-dimethylformamide 2mL was added to 25 mL bottom and stirred at 80 °C for 24 hours.20mL water was added into the bottom, KPF_6 saturated solution was added to the solution, filtered, the precipitate was dissolved in CH_3CN. The CH_3CN solution was added to a solution of excess tetrabutyl ammonium chloride in CH_3CN, filtered, the residue was dissolved in CH_3OH 200 uL excess ethylacetate was added to the solution, filtered, the precipitate was dryed in vacuum oven.The product compound *OTPP-6-Ethyl* was obtained as a yellow solid (77mg, yield: 95%).^{1}H NMR (400 MHz, CD_3OD) δ: 1.68-1.72(m,18H),4.64-4.94(m,12H),7.42-7.44(d,18H,J=8),7.77-7.82(m,6H),8.09-8.12(d,12H,J=12),8.42-8.44(d,12H,J=8),8.96-8.98(d,12H,J=8), ppm. ^{13}C NMR (100 MHz, CD_3OD) δ:155.08,149.80,144.01,129.62,129.20,125.06,123.94,55.99,15.25 ppm. MALDI-TOF (m/z): Calcd. For $[C_{96}H_{90}N_9OP]^{6+}$ (M/Z, Z=5)283.14, found 283.34.

Synthesis of Compound OTPP-6-Propyl

OTPP-3-BPA (62mg,0.05mmol), 1-bromopropane (123mg,1mmol), N,N-dimethylformamide 2mL was added to 25 mL bottom and stirred at 80 °C for 24 hours.20mL water was added into the bottom, KPF_6 saturated solution was added to the solution, filtered, the precipitate was dissolved in CH_3CN. The CH_3CN solution was added to a solution of excess tetrabutyl ammonium chloride in CH_3CN, filtered, the residue was dissolved in CH_3OH 200 uL excess ethylacetate was added to the solution, filtered, the precipitate was dryed in vacuum oven.The product compound *OTPP-6-Propyl* was obtained as a yellow solid (84mg, yield: 98%).^{1}H NMR (400 MHz, CD_3OD) δ: 1.02-1.09(m,18H),2.07-2.12(m,12H),4.53-4.67(m,12H),7.42-7.44(d,18H,J=8),7.77-7.82(m,6H),8.10-8.12(d,12H,J=8),8.42-8.44(d,12H,J=8),8.95-8.97(d,12H,J=8) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ:155.12,149.82,144.28,133.33,129.64,129.17,125.05,123.86,61.95,24.31,9.38 ppm. MALDI-TOF (m/z): Calcd. For $[C_{102}H_{102}N_9OP]^{6+}$ (M/Z, Z=5)299.96, found 300.16.

Synthesis of Compound OTPP-6-Amyl

OTPP-3-BPA (62mg,0.05mmol), 1-bromopentane (151mg,1mmol), N,N-dimethylformamide 2mL was added to 25 mL bottom and stirred at 80 °C for 24 hours.20mL water was added into the bottom, KPF_6 saturated solution was added to the solution, filtered, the precipitate was dissolved in CH_3CN. The CH_3CN solution was added to a solution of excess tetrabutyl ammonium chloride in CH_3CN, filtered, the residue was dissolved in CH_3OH 200 uL excess ethylacetate was added to the solution, filtered, the precipitate was dryed in vacuum oven.The product compound *OTPP-6- Amyl* was obtained as a yellow solid (89mg, yield: 95%).^{1}H NMR (400 MHz, CD_3OD) δ: 0.97-1.00 (m, 18H), 1.36-1.49 (m, 24H) 2.05-2.09 (m, 12H), 4.60-4.64 (m, 12H), 7.42-7.44(d,18H,J=8),7.77-7.82(m,6H),8.10-8.12(d,12H,J=8),8.42-8.44(d,12H,J=8),8.95-8.97(d,12H,J=8), ppm. ^{13}C NMR (100 MHz, CD_3OD) δ: 155.08,149.80,144.01,133.44,129.62,129.20,125.06,123.94,55.99,15.25 ppm. MALDI-TOF (m/z): Calcd. For $[C_{114}H_{126}N_9OP]^{6+}$ (M/Z, Z=5)333.60, found 333.99.

Synthesis of Compound OTPP-5-M-1-Mal

OTPP-3-BPA (620mg,0.5mmol), 1-(4-(bromomethyl)phenyl)-1H-pyrrole-2,5-dione(26.5mg,0.1mmol), N,N-dimethylformamide 5mL was added to 25 mL bottom and stirred at 80 °C for 48 hours. N,N-dimethylformamide was removed under vacuum. The excess OTPP-3-BPA was recycled by column chromatography (SiO2, EtOAc-Methol5:1).Then the mixture and silica gel was derectly react with CH_3I at room temperature. CH_3I was removed in vacuo, the mixture was dissovled into water, KPF_6 saturated solution was added to the solution, and the CH_3CN solution was added to a solution of excess tetrabutyl ammonium chloride in CH_3CN, filtered, the residue was dissolved in CH_3OH 200 uL excess ethylacetate was added to the solution, filtered, the precipitate was dryed in vacuum oven.The product compound *OTPP-5-M-1-Mal* was obtained as a yellow solid (20mg, yield: 12%) ^{1}H NMR (400 MHz, CD_3OD) δ: 4.41 (s, 15H), 5.78(s,2H),7.22-7.47(m,18H),7.52-7.60(m,4H),7.56-7.85(m,8H),8.09-8.11(d,12H,J=8),8.40-8.41(d,12H,J=4),8.87-8.89(d,10H,J=8),9.01(s,2H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ:154.76,149.76,145.17,133.48,129.58,129.15,125.03,124.54,123.56 ppm MALDI-TOF (m/z): Calcd. For $[C_{100}H_{83}N_{10}O_3P]^{6+}$ (M/Z, Z=3)500.88, found ((M+3K)/Z, Z=3)540.53.

Synthesis of Compound OTPP-5-M-1-cRGD

OTPP-5-M-1-Mal(1.72mg,1uMol),cRGD(0.7mg, 1.2uMol), CH3OH/Water (1:1, 1mL),was added to 2mL PE centrifuge tube and stirred at room temperature over night. CH3OH was removed, The mixture was dissolved into 0.5ml water. Potassium hexafluorophosphate was added to the solution and the precipitate was dissolved in acetonitrile. The acetonitrile solution was added to a solution of excess tetrabutyl ammonium chloride in acetonitrile and the deposit was filtered and washed with acetonitrile for five times. By drying the solid in a vacuum oven, desired product compound *OTPP-5-M-1-cRGD* was obtained as a yellow solid (2.0mg, 87%). Because of its complex structure and small amount, it is difficult to analyze by NMR. its generation can be identified by HRMS in the presence of related peak. MALDI-TOF (m/z): Calcd. For $[C_{124}H_{1117}BN_{18}O_{10}PS]^{6+}$ (M/Z, Z=2) 1041.22, found((M+6H)/Z, Z=2) 1044.99; ((M+2Cl)/Z, Z=2) 1077.02; ((M+4Cl)/Z, Z=2) 1109.05; ((M+2Cl+4Na)/Z, Z=2) 1122.97; ((M+2Cl+2K)/Z, Z=2) 1154.99.

3. Cell culture and imaging

Human umbilical vein endothelial cells (HUVEC-1) were cultured in Bronchial Epithelial Cell Growth Medium supplemented with 10% fetal bovine serum (FBS). Other cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with glucose (4.5 g/L), L-glutamine, sodium pyruvate, and 10% fetal bovine serum (FBS). The cells were plated on glass bottomed dishes at 37 °C under 5% CO2 atmosphere before imaging. Apoptotic cells were obtained by culturing in PBS for another 12-24 hours before imaging. Cell imaging were conducted using a confocal microscope FV1000-IX81 and were analyzed with FV10-ASW software.Cells, pre-washed twice, were incubated with various probes in cultured medium without FBS at 37°C under 5% CO2 for corresponding time. Then the cells were washed with PBS to remove unbounded probes for six times before in situ imaging by Olympus Fluorescence confocal microscope.

4. ROS detection

The DMSO solution of H2DCF-DA (2.0 mM, 100 μL) was activated by sodium hydroxide solution (0.01 M, 0.8 mL) and allowed to sit at room temperature for 30 min, which was added to 4.1 mL PBS with the DMSO solution of probe (1.0 mM, 50.0 μL) a half an hour later in dark. The fluorescence signal was monitored after the solution was irradiated by LED light (1.5 mW/cm2) and the fluorescence spectra were recorded with the excitation wavelength at 480 nm.

SKOV-3 and U87MG cells in confocal culture dishes were preloaded with OTPP-6-Amyl and OTPP-5-M-1-cRGD, respectively. Then, the cells were treated with H2DCF-DA (5 μM) aqueous solution and incubated for 20 min in darkness, wrapped in foil in a 37 0C cell incubator. Next, the cells were irradiated by the LED light (1.5 mW/cm^2). Detection of H2DCF-DA fluorescence was visualized by confocal microscope. H2DCF-DA was excited by a 488 nm laser, and fluorescence emission at 490-530 nm was recorded by confocal laser scanning microscopy using oil objective.

5. Tumor model and In vivo imaging

Nude mice 6-7 weeks old were provided by the Laboratory Animal Center of North Sichuan Medical College, Nanchong, China. All procedures involving animals were performed according to a protocol approved by **the Institutional Animal Care and Treatment Committee of North Sichuan Medical College**. These nude mice were subcutaneously injected with 1×106SKOV-3 cells or U87MG cells in the left anterior axillary under aseptic conditions. Then they were individually housed under specific pathogen-free conditions with free access to food and water until the formed tumor grow to approximately 0.5cm in diameter by measuring caliper; tumor growth to this size took about a month. These tumor-bearing mice were fasting for 24h and then were anesthetized by intraperitoneal injection of 0.05mL 3% aqueous solution of pentobarbital. The mice were then placed into the small animal imager and injected intraperitoneally with a certain amount of probes solution for imaging.

Results and Discussion

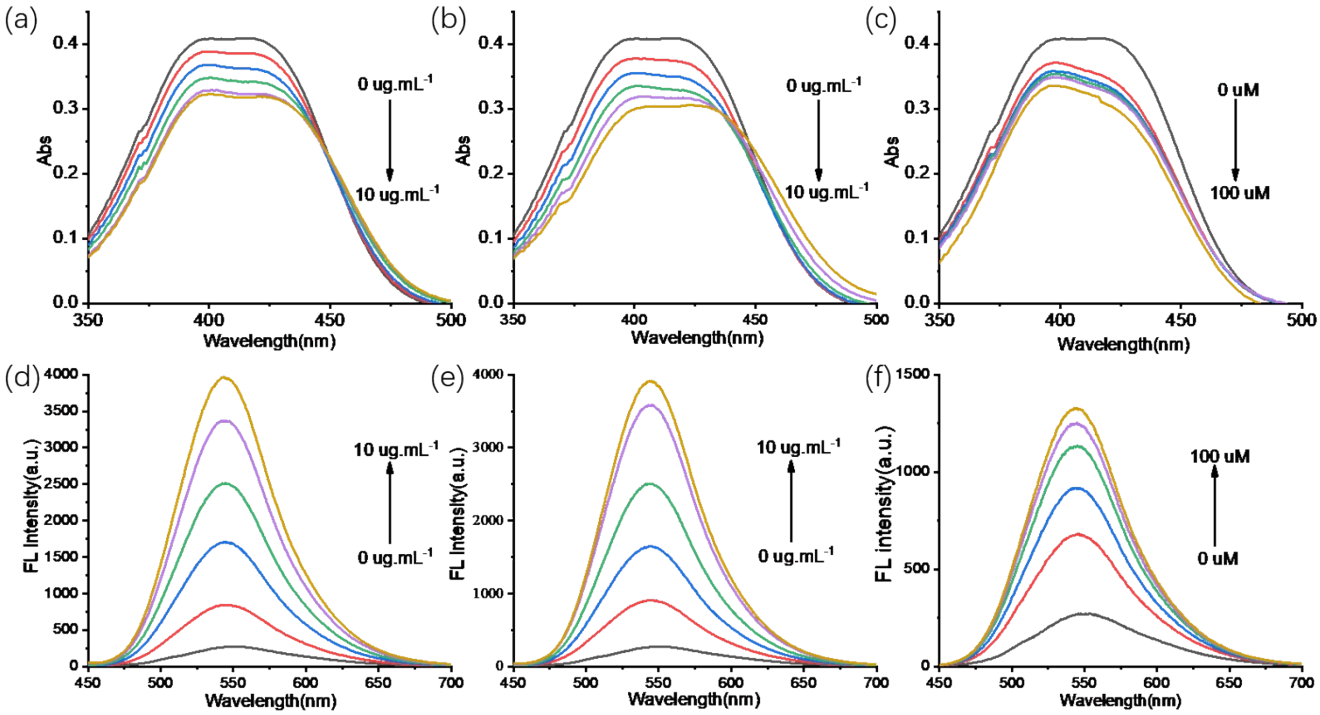


Figure S1. Absorption spectra (a, b, c), and fluorescence spectra (d, e, f), showing changes of OTPP-6-Ethyl (5 μM) in water with the addition of different amounts of DNA (a, d), RNA (b, e) and ATP (c, f). Ex: 430 nm.

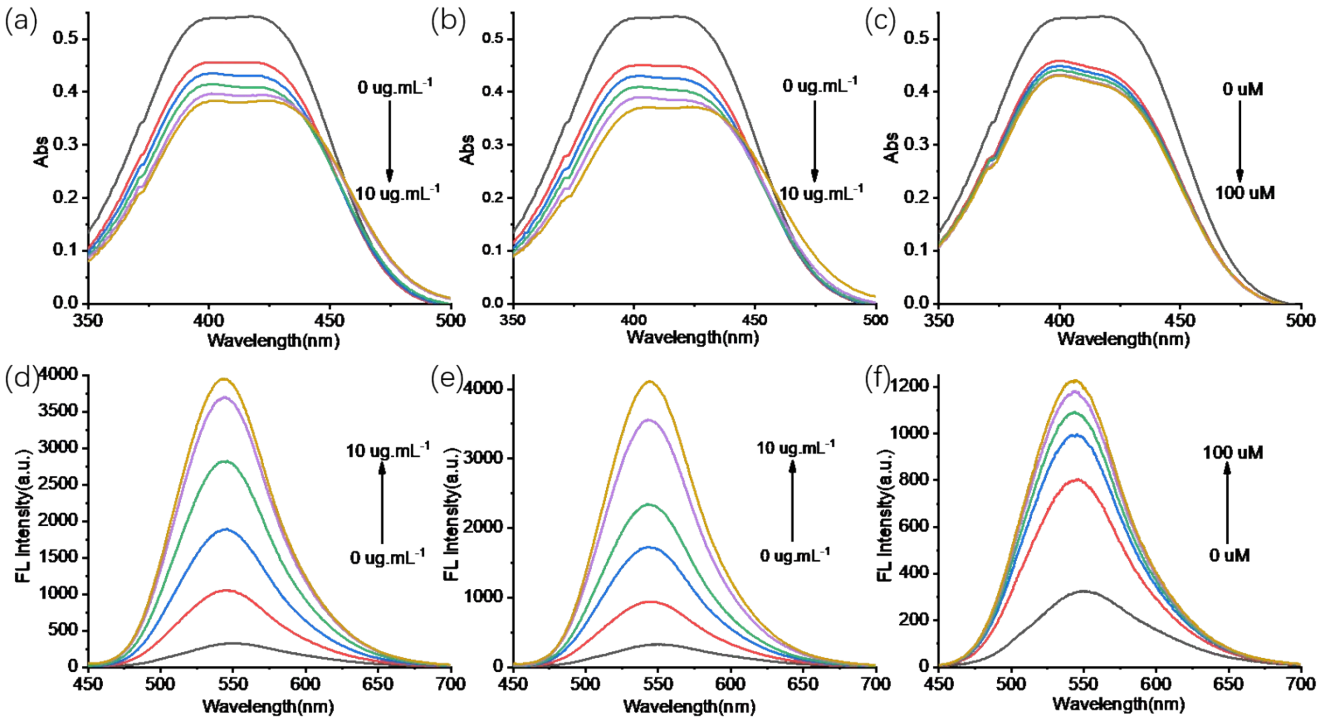


Figure S2. Absorption spectra (a, b, c), and fluorescence spectra (d, e, f), showing changes of OTPP-6-Propyl (5 μM) in water with the addition of different amounts of DNA (a, d), RNA (b, e) and ATP (c, f). Ex: 430 nm.

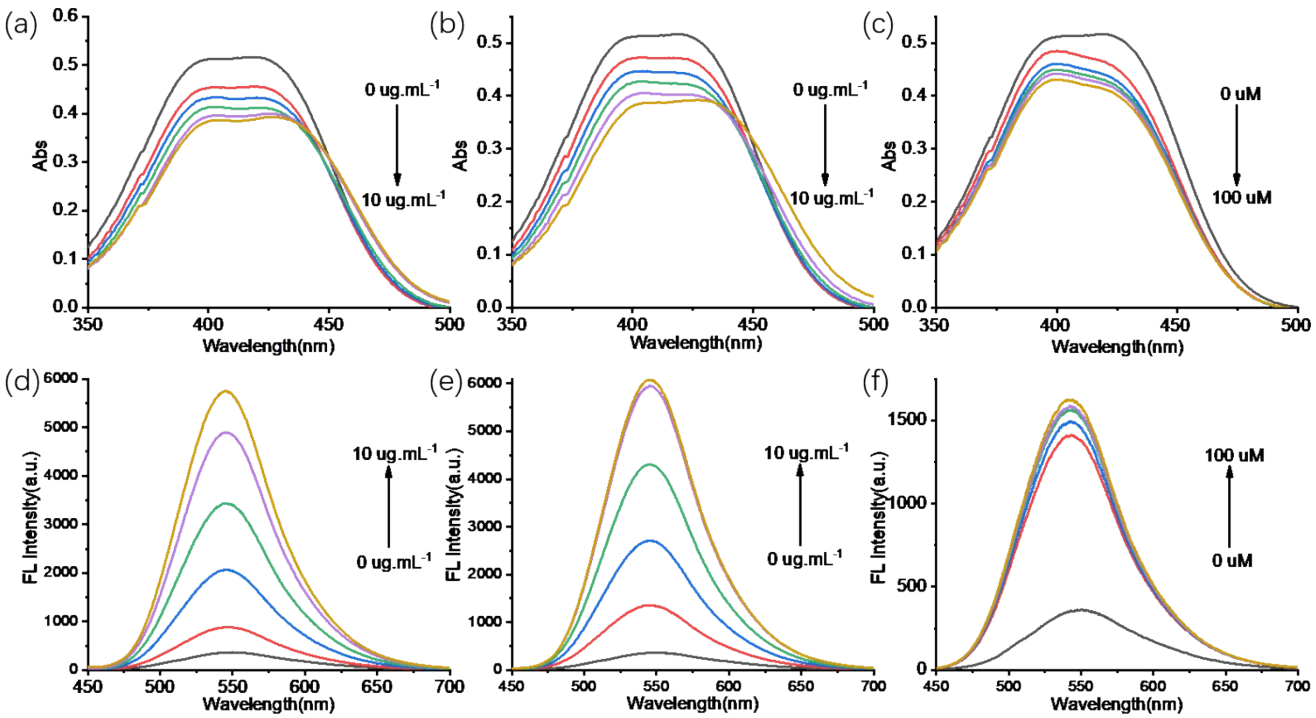


Figure S3. Absorption spectra (a, b, c), and fluorescence spectra (d, e, f), showing changes of OTPP-6-Amyl (5 μM) in water with the addition of different amounts of DNA (a, d), RNA (b, e) and ATP (c, f). Ex: 430 nm.

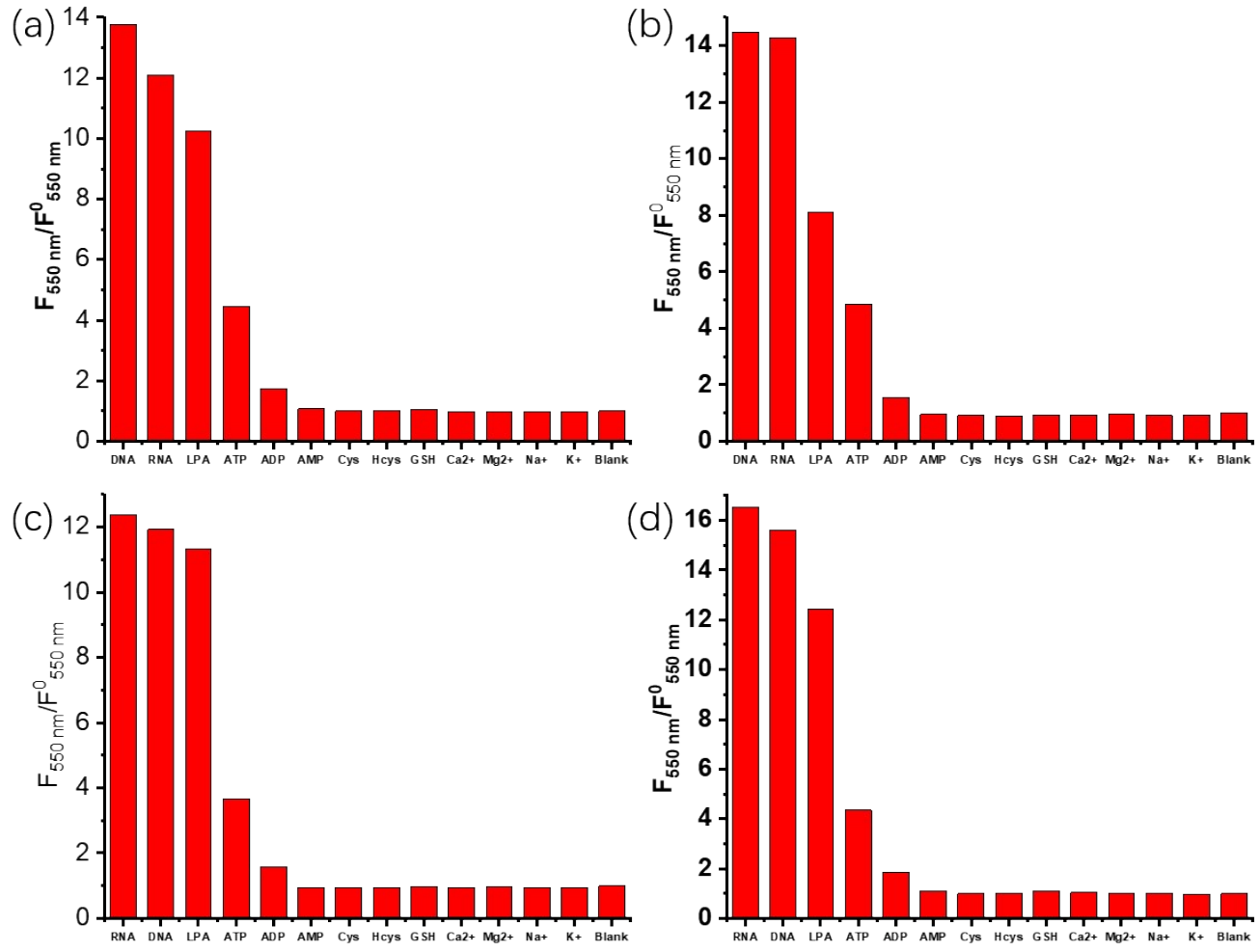


Figure S4. Fluorescence responses of (a) OTPP-6-Methyl, (b) OTPP-6-Ethyl, (c) OTPP-6-Propyl and (d) OTPP-6-Amyl to various substances.

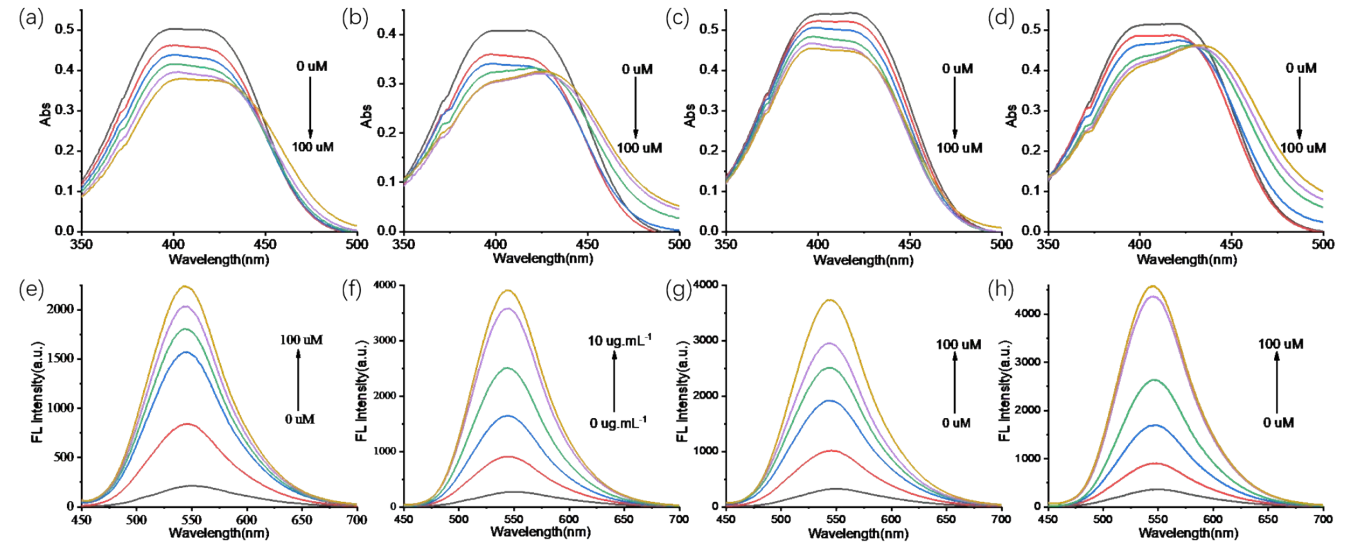


Figure S5. (a-d) UV absorption and (e-h) fluorescence spectral changes of 5μM OTPP-6-Methyl (a,e), OTPP-6-Ethyl (b, f), OTPP-6-Propyl (c, g), (d, h) in water (containing 0.5% DMSO) upon the addition of LPA. Ex: 430 nm.

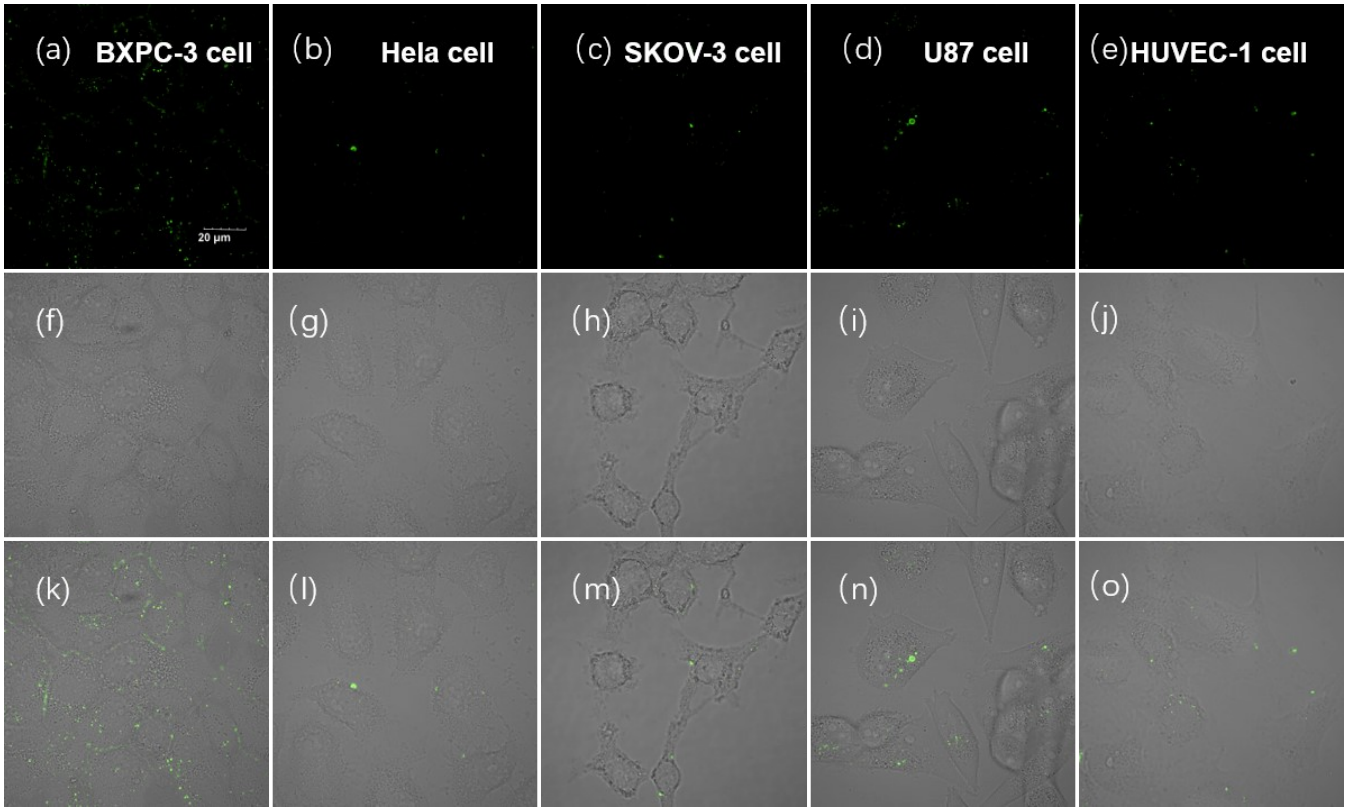


Figure S6. (a-e) Fluorescence images of OTPP-6-Methyl (5μM) staining living BXPC-3, Hela, HUVEC-1, U87MG and SKOV-3 cells. (f-j) are the corresponding bright field images and (k-o) are the overlay images.

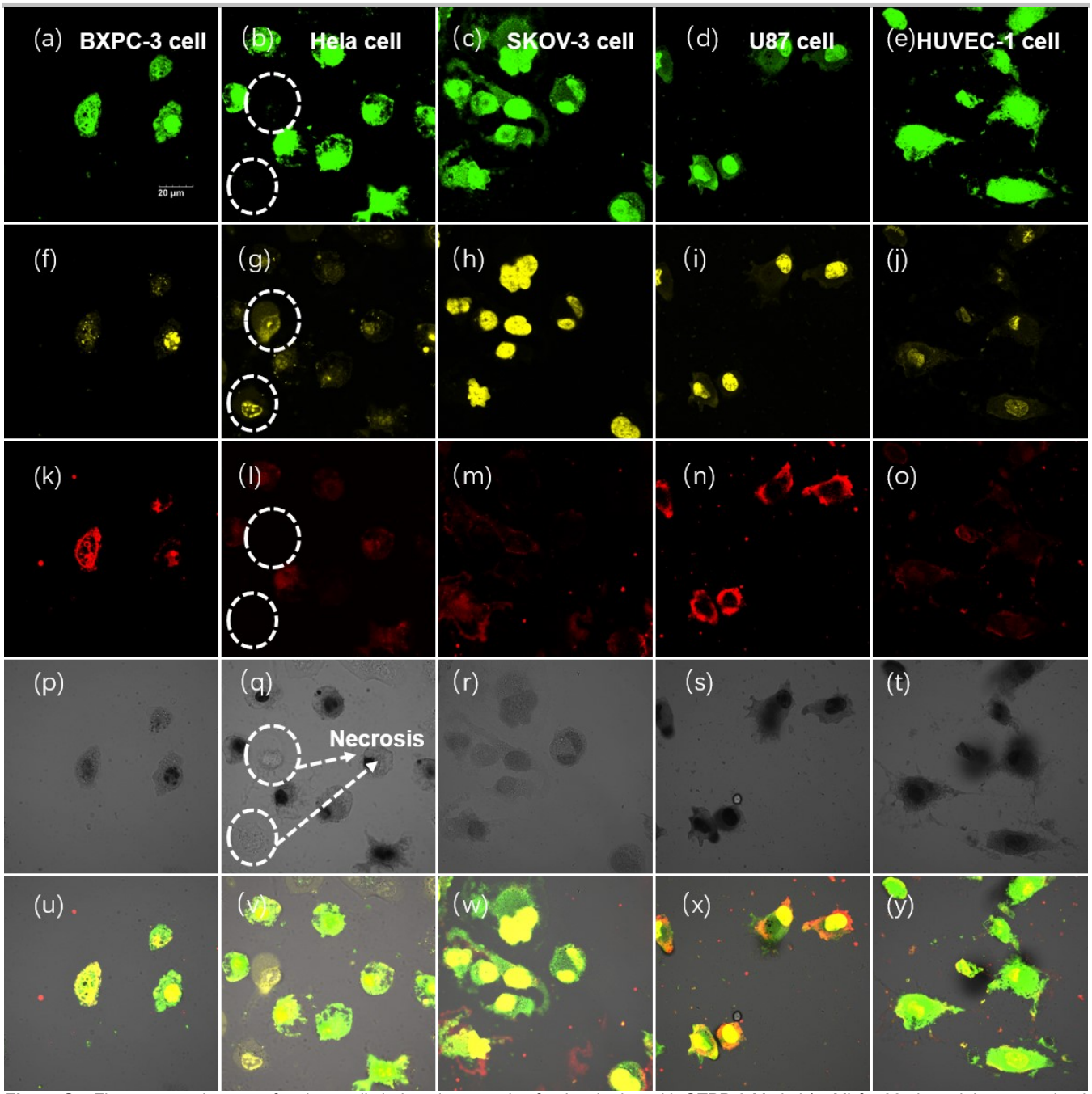


Figure S7. Fluorescence images of various cells induced apoptosis after incubating with OTPP-6-Methyl (5μM) for 30min and then co-stained with AV 647/PI under excitation at 405 nm, 560 and 640 nm, respectively. (a-e) OTPP-6-Methyl collected from 530 to 580nm; (f-j) PI collected from 570 to 670nm; (k-o) AV647 collected from 650-750nm, respectively. (p-t) are their bright field images and (u-y) are their overlay images.

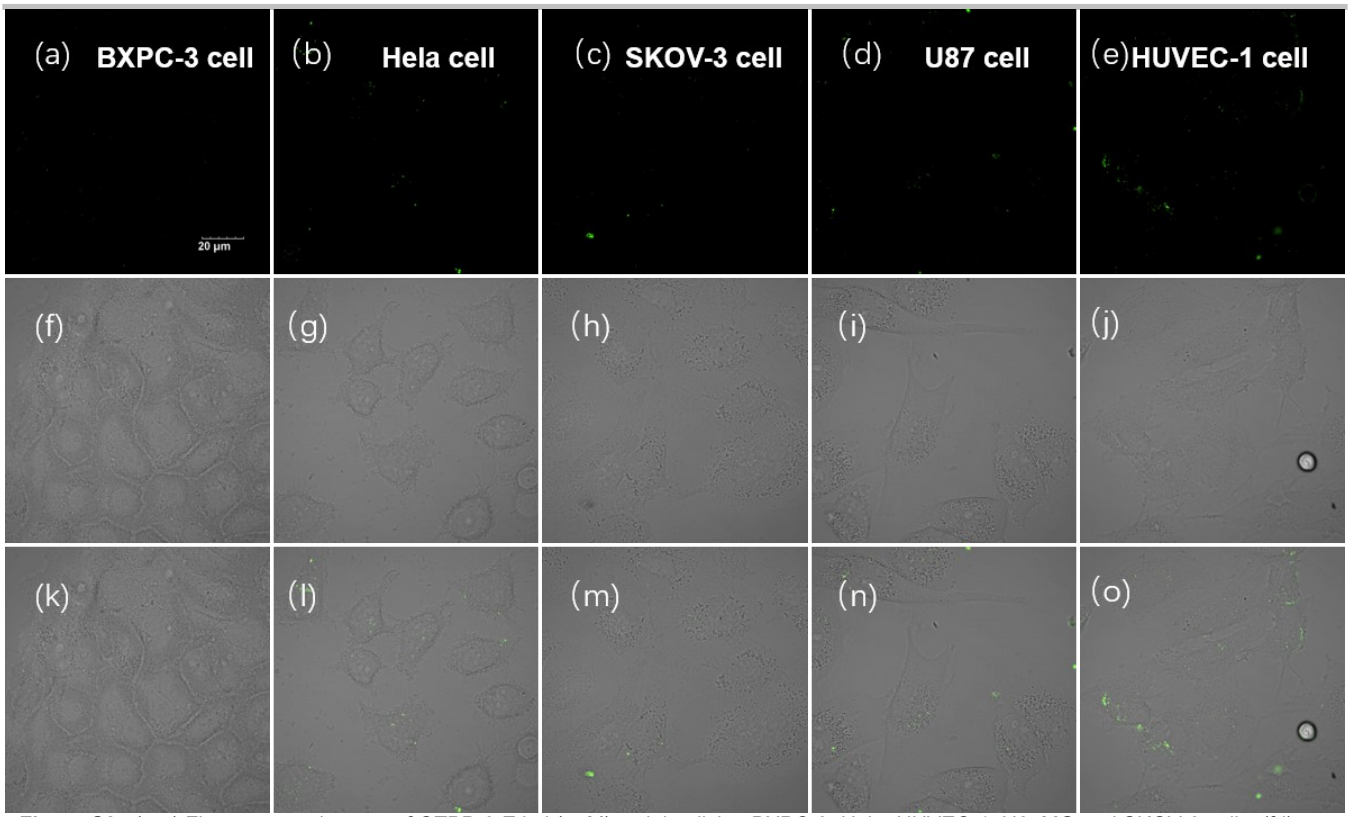


Figure S8. (a-e) Fluorescence images of OTPP-6-Ethyl (5μM) staining living BXPC-3, Hela, HUVEC-1, U87MG and SKOV-3 cells. (f-j) are the corresponding bright field images and (k-o) are the overlay images.

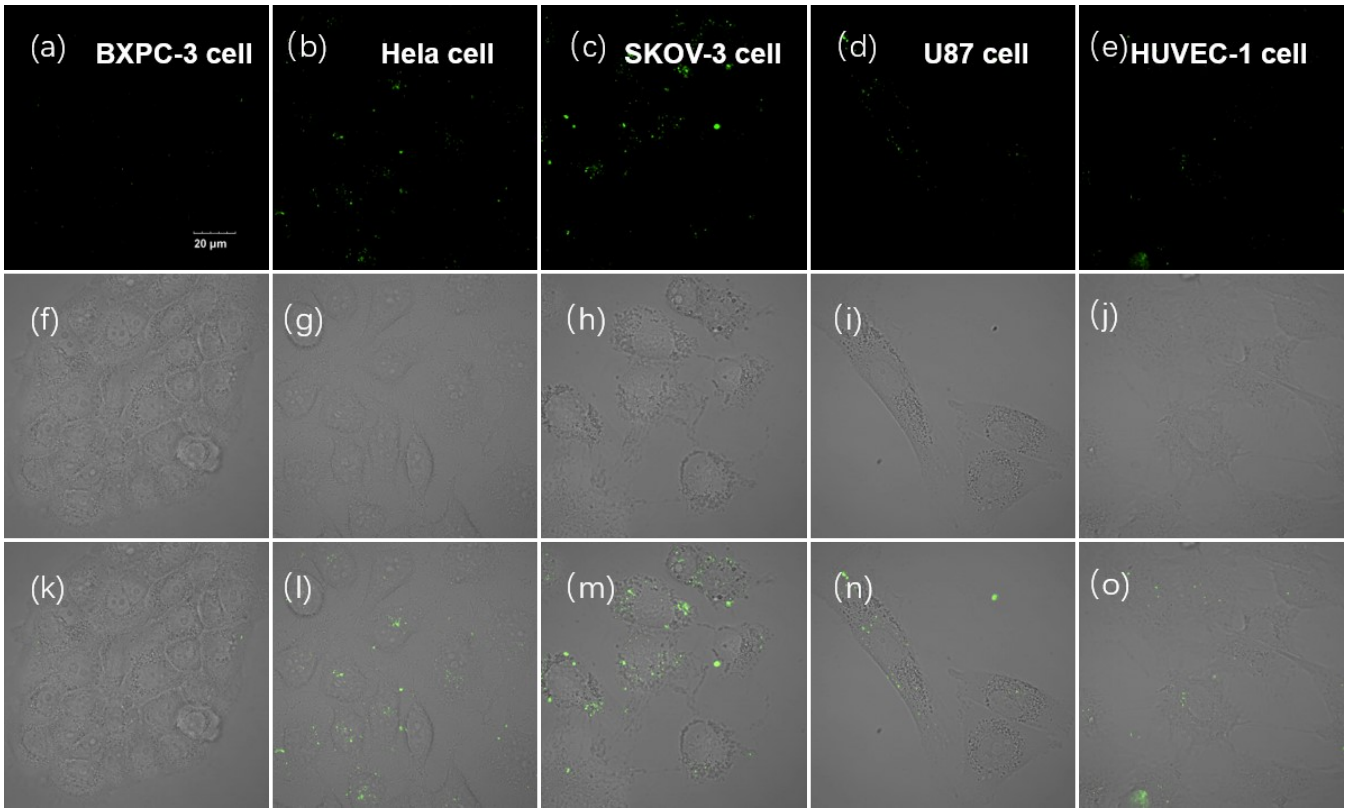


Figure S9. (a-e) Fluorescence images of OTPP-6-Propyl (5μM) staining living BXPC-3, Hela, HUVEC-1, U87MG and SKOV-3 cells. (f-j) are the corresponding bright field images and (k-o) are the overlay images.

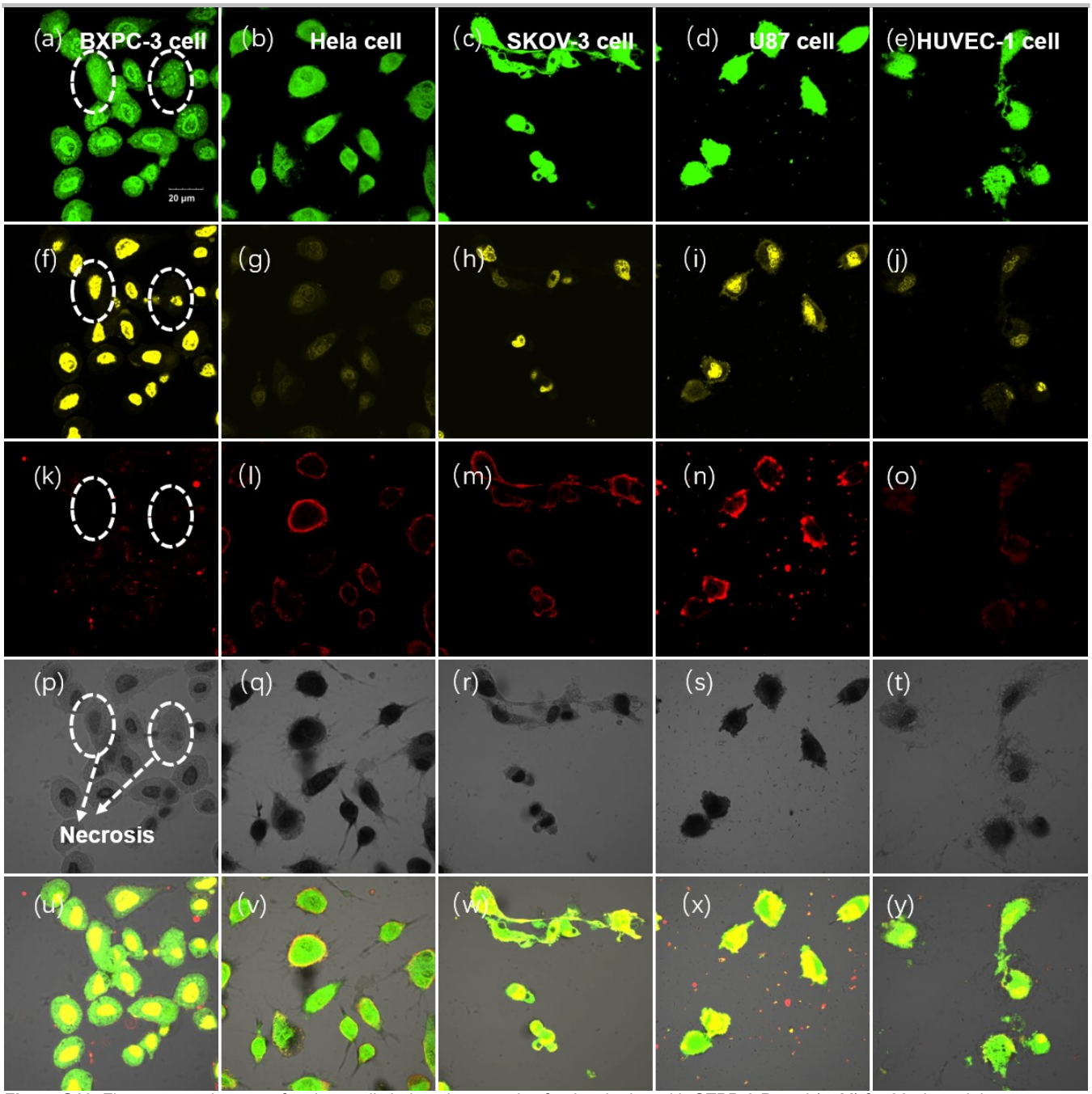


Figure S10. Fluorescence images of various cells induced apoptosis after incubating with OTPP-6-Propyl (5μM) for 30min and then co-stained with AV 647/PI under excitation at 405 nm, 560 and 640 nm, respectively. (a-e) OTPP-6-Propyl collected from 530 to 580nm; (f-j) PI collected from 570 to 670nm; (k-o) AV647 collected from 650-750nm, respectively. (p-t) are their bright field images and (u-y) are their overlay images.

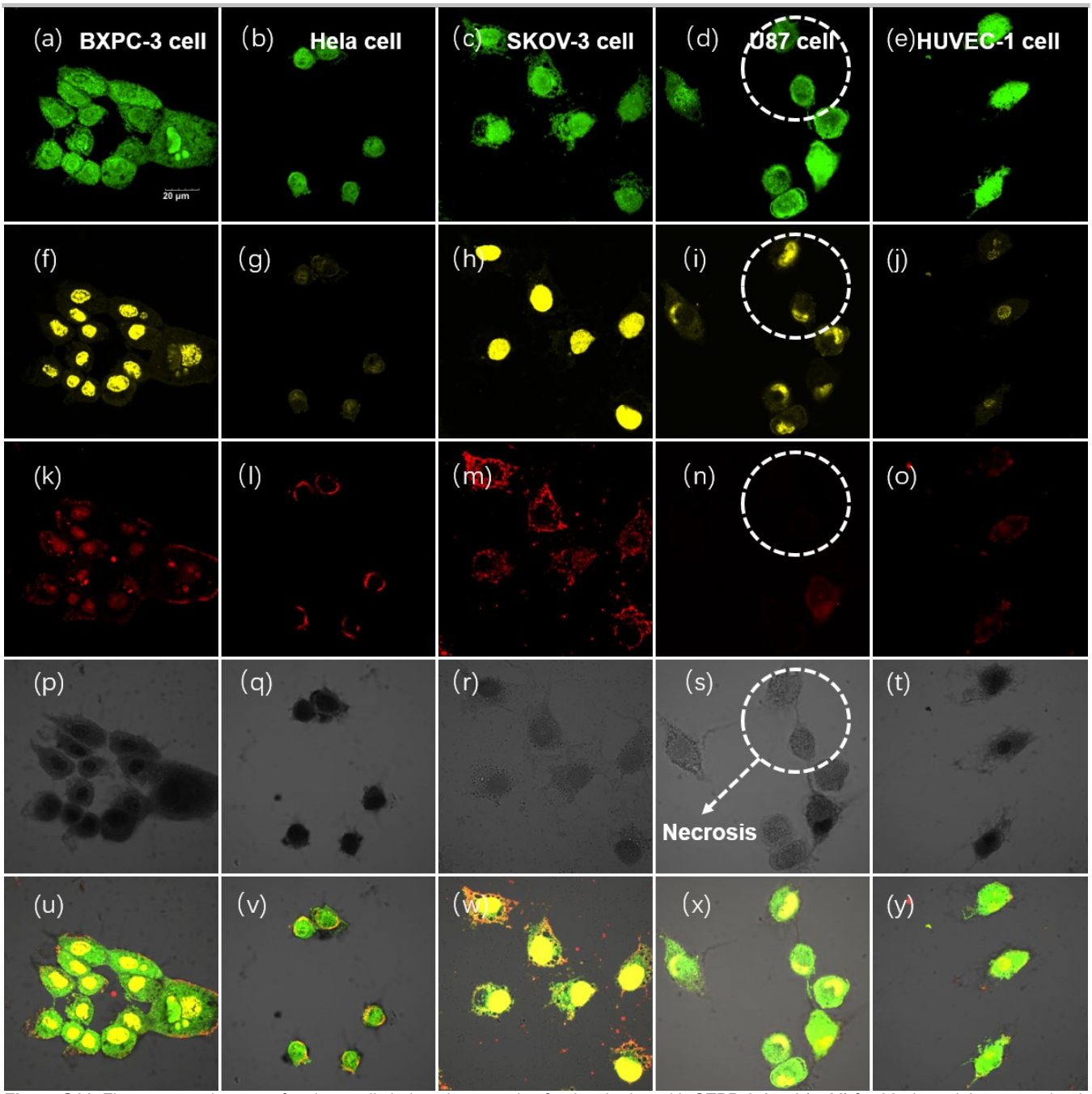


Figure S11. Fluorescence images of various cells induced apoptosis after incubating with OTPP-6-Amyl (5μM) for 30min and then co-stained with AV 647/PI under excitation at 405 nm, 560 and 640 nm, respectively. (a-e) OTPP-6-Amyl collected from 530 to 580nm; (f-j) PI collected from 570 to 670nm; (k-o) AV647 collected from 650-750nm, respectively. (p-t) are their bright field images and (u-y) are their overlay images.

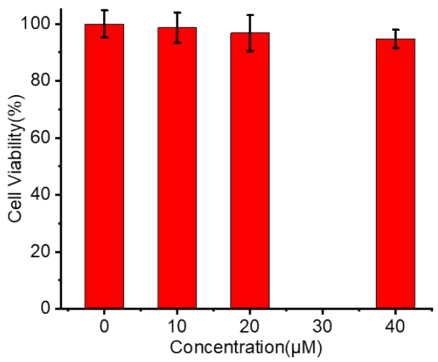


Figure S12. Cytotoxicity of OTPP-6-Amyl on Hela cells determined by MTT assay.

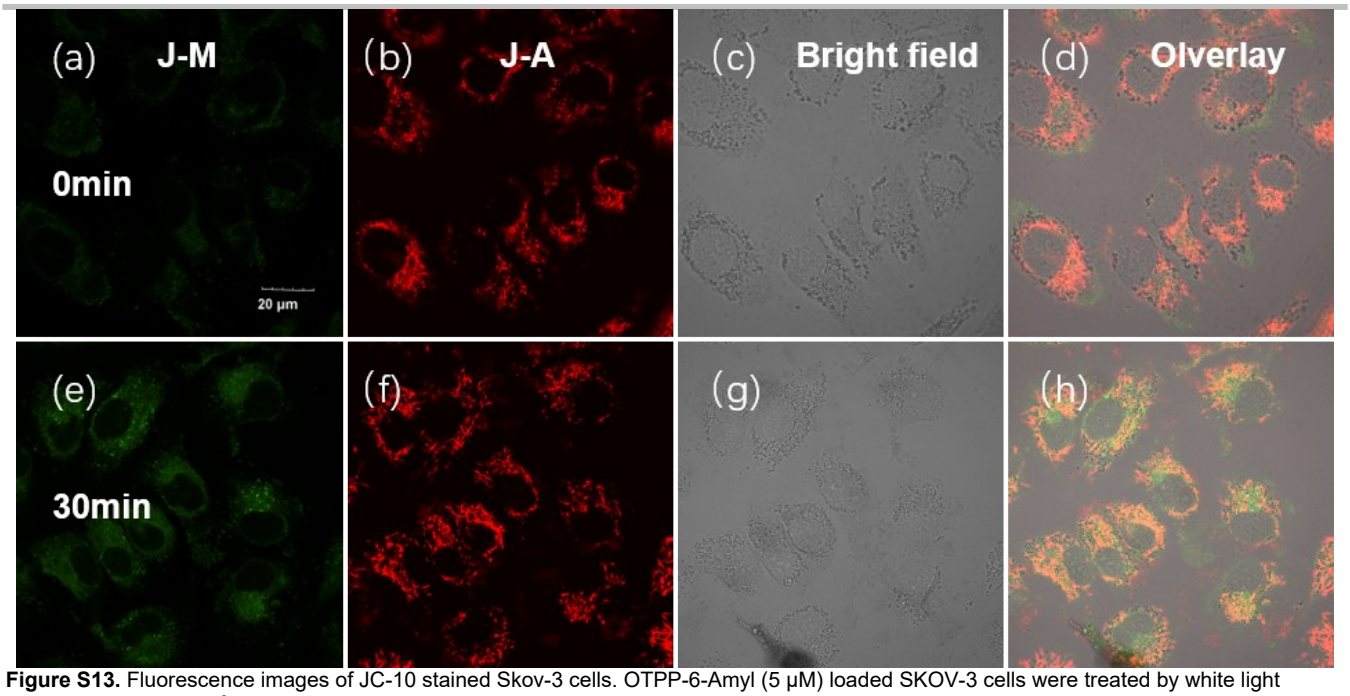


Figure S13. Fluorescence images of JC-10 stained Skov-3 cells. OTPP-6-Amyl (5 μM) loaded SKOV-3 cells were treated by white light irradiation (1.5 mW/cm^2) for 30 min. Cells were viewed in the green channel for JC-10 monomers (λ_{ex} = 488 nm, λ_{em} = 520-550 nm) and the red channel for J-aggregates (λ_{ex} = 488 nm, λ_{em} = 600-640 nm). J-A and J-M stand for the J-aggregates and J-monomers.

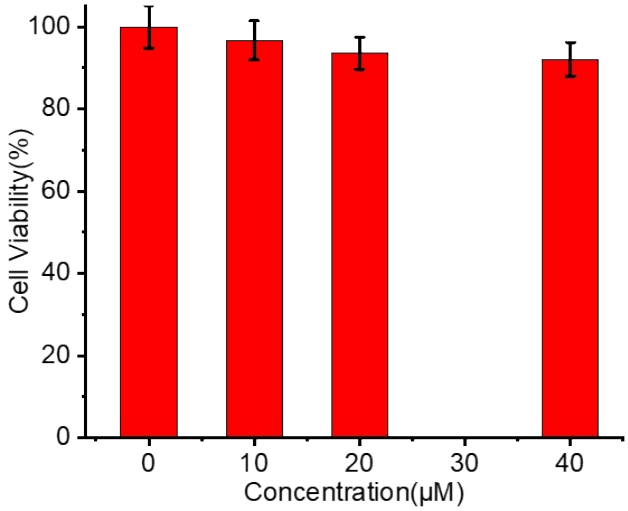


Figure S14. Cytotoxicity of OTPP-5-M-1-cRGD on Hela cells determined by MTT assay.

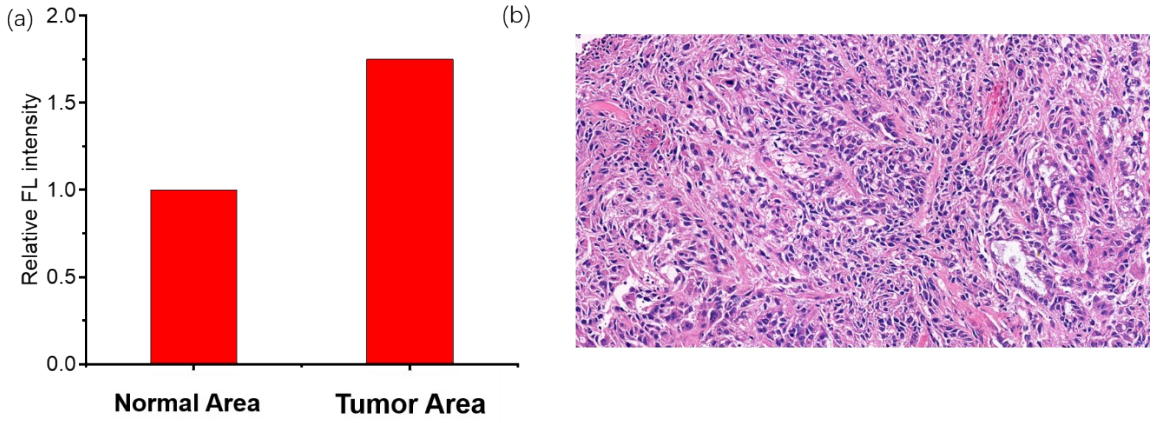


Figure S15. (a) Relative fluorescence intensity of U87MG tumor and non tumor sites; (b) tumor H&E-stained slices of the mice.

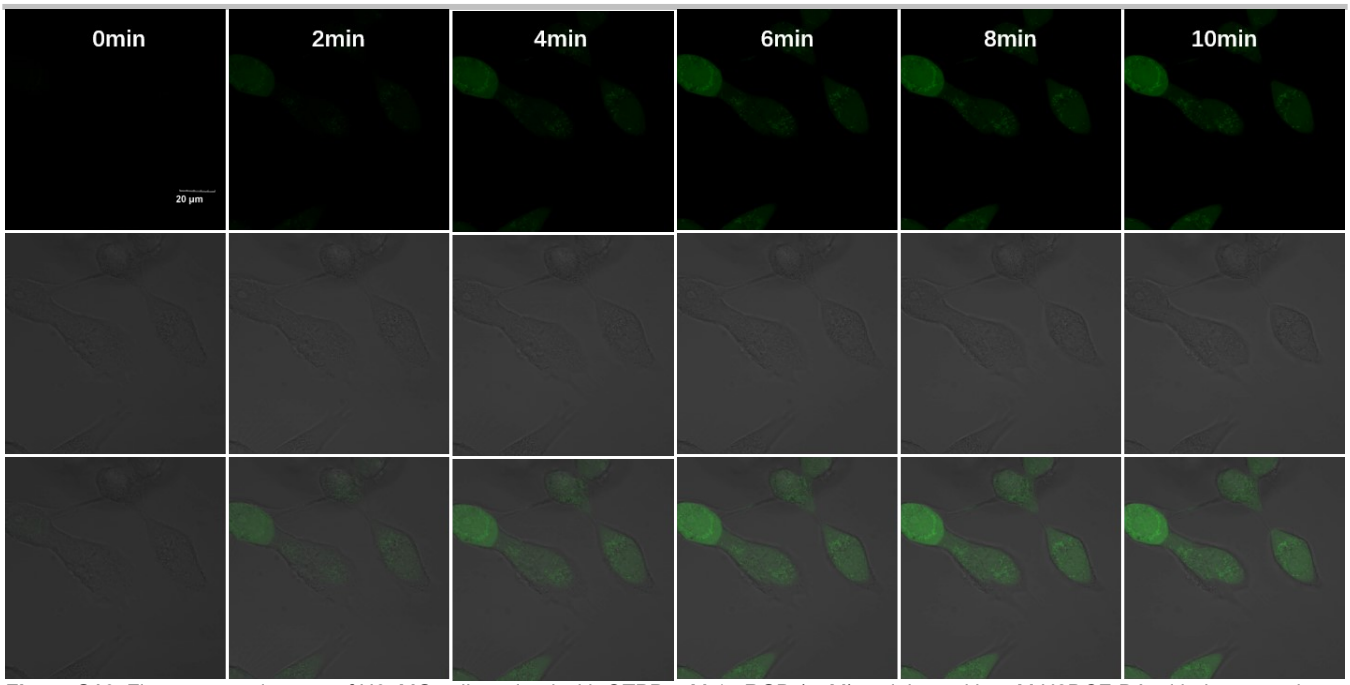


Figure S16. Fluorescence images of U87MG cells stained with OTPP-5-M-1-cRGD (5μM) and then with 5uM H2DCF-DA with the extension of light irradiation (1.5mW/cm2) time.

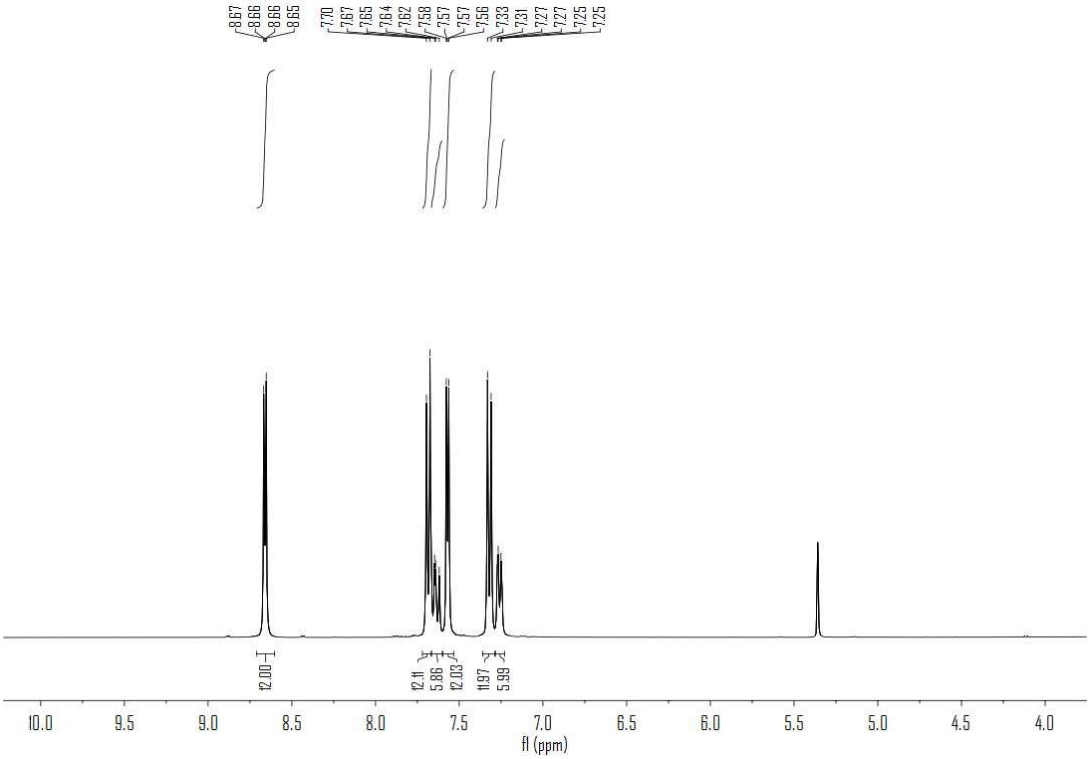


Figure S17. ^{1}HNMR spectra of OTPP-3-BPA.

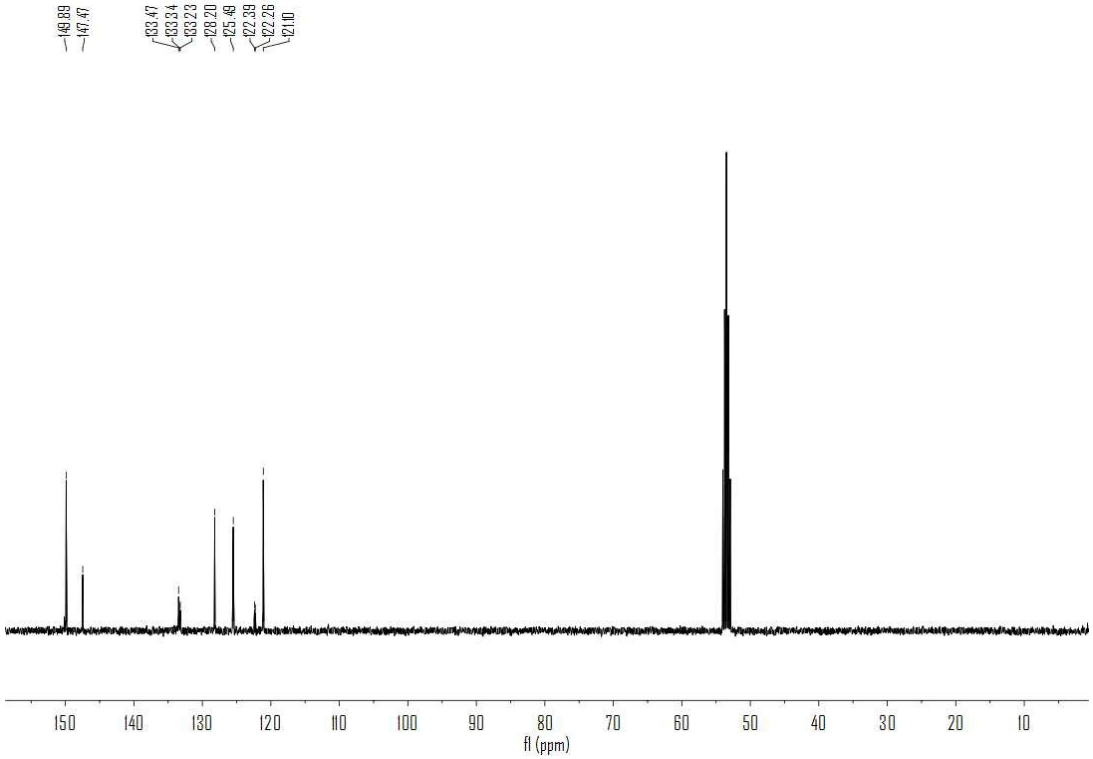


Figure S18. ^{13}CNMR spectra of OTPP-3-BPA.

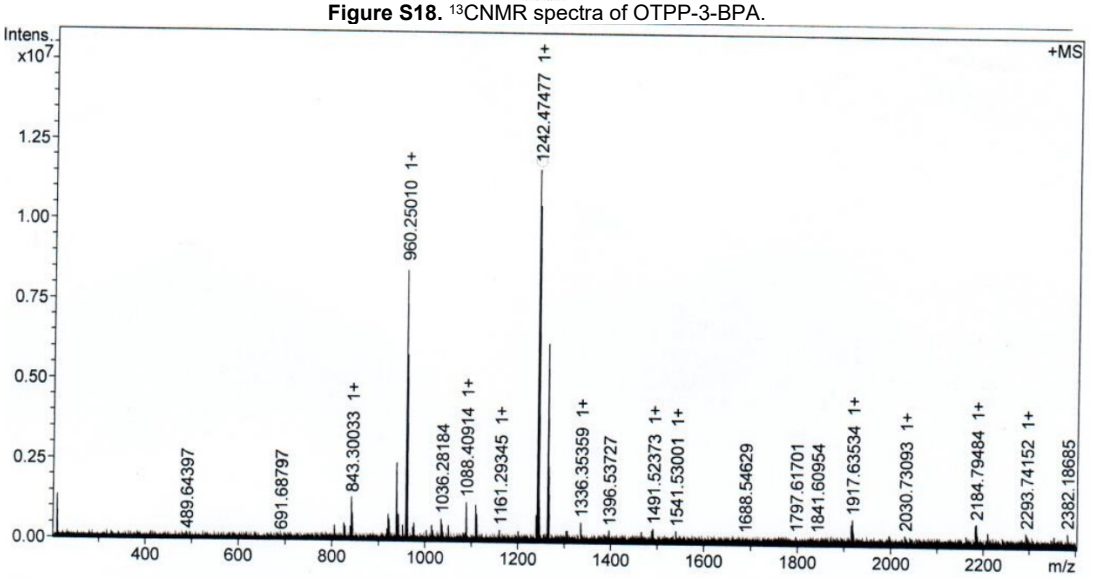


Figure S19. HRMS spectra of OTPP-3-BPA.

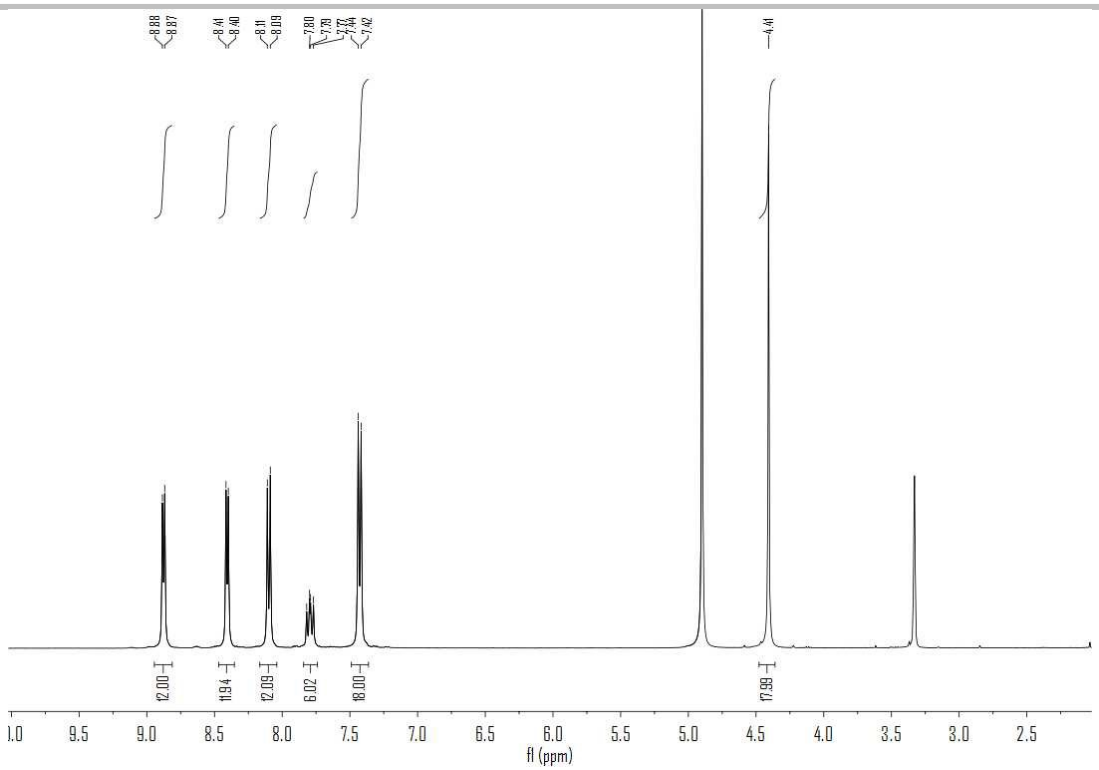


Figure S20. [1]HNMR spectra of OTPP-6-Methyl.

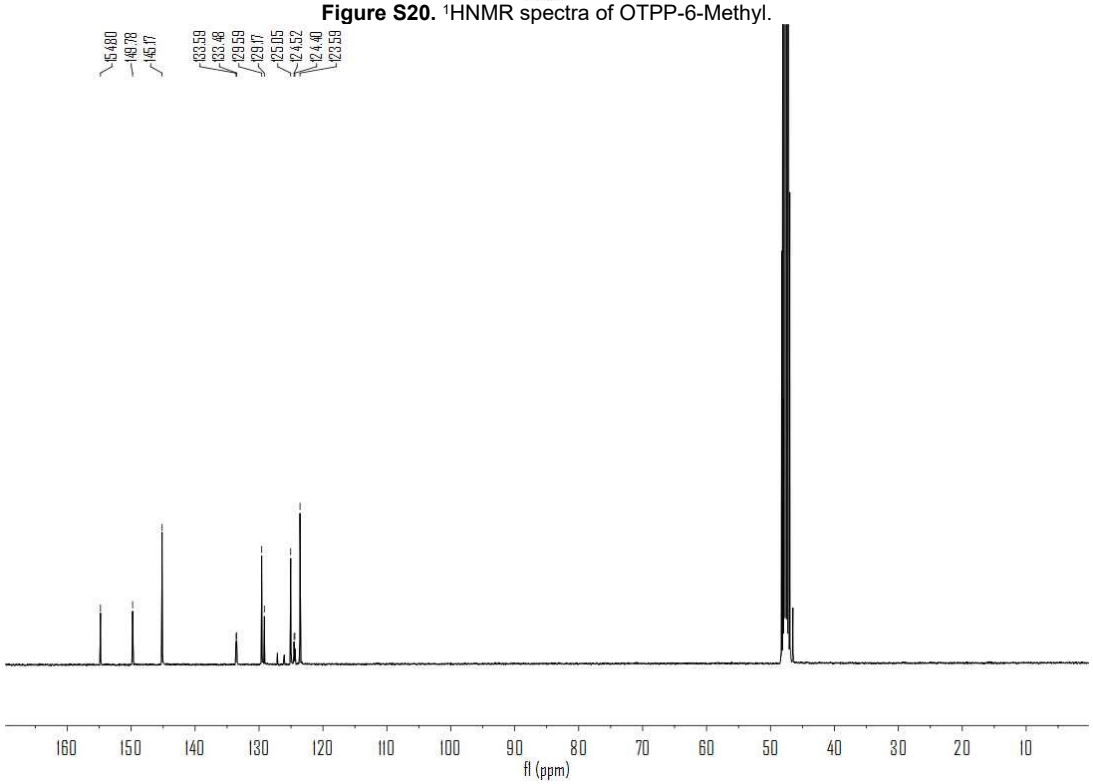


Figure S21.[13]CNMR spectra of OTPP-6-Methyl.

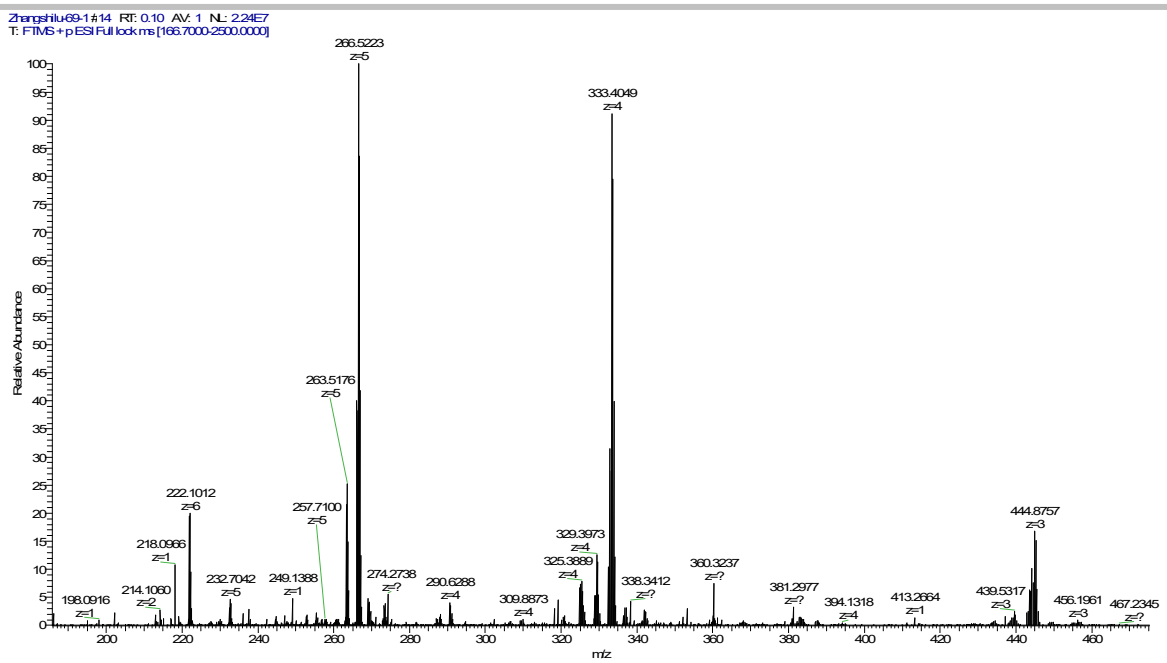


Figure S22. HRMS spectra of OTPP-6-Methyl.

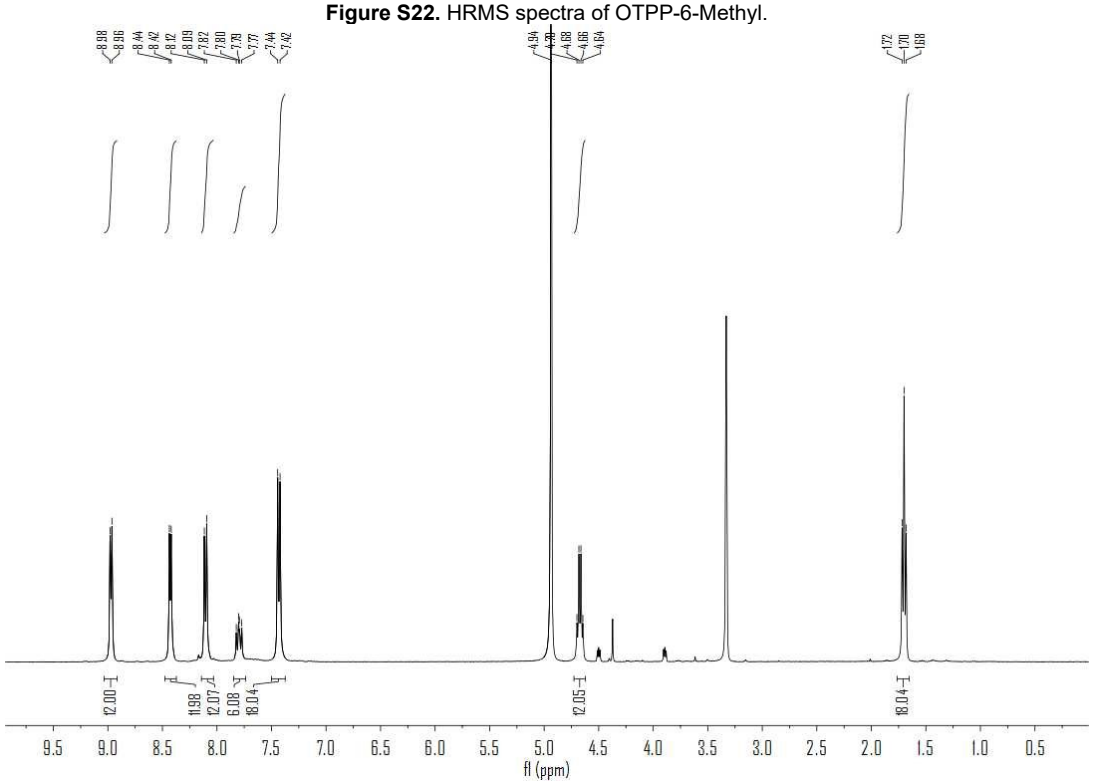


Figure S23. ^{1}HNMR spectra of OTPP-6-Ethyl.

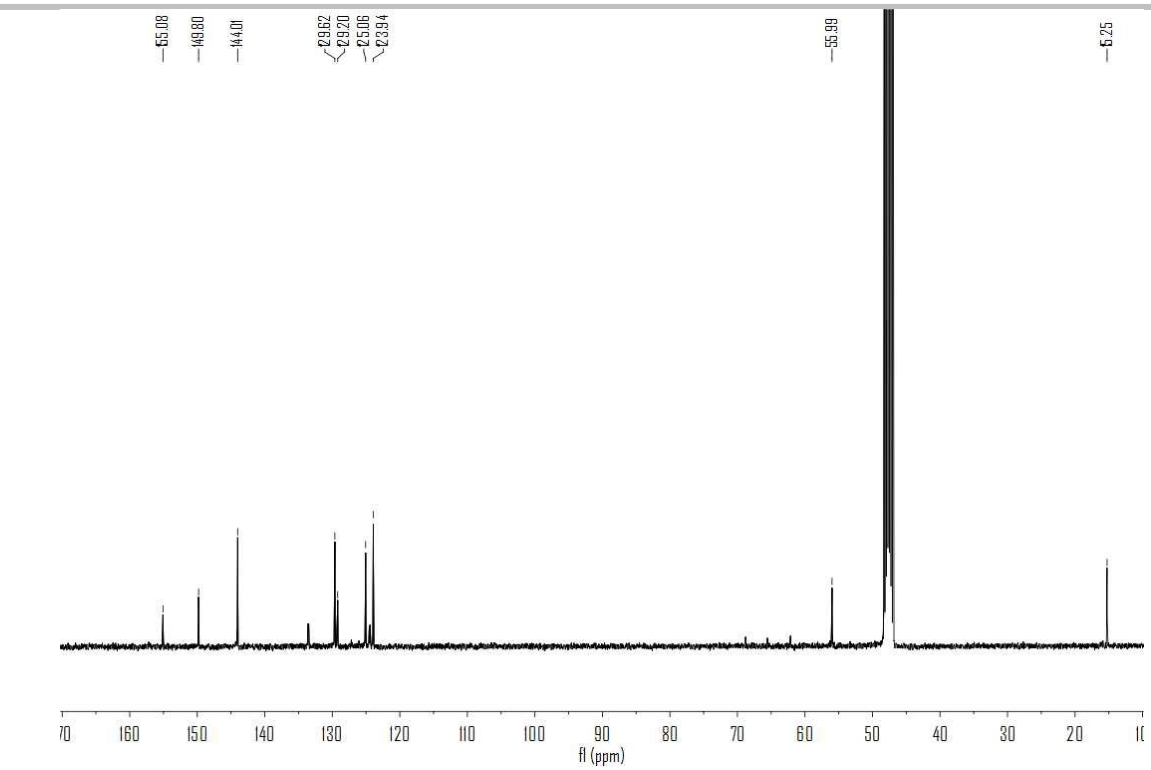


Figure S24. ^{13}CNMR spectra of OTPP-6-Ethyl.

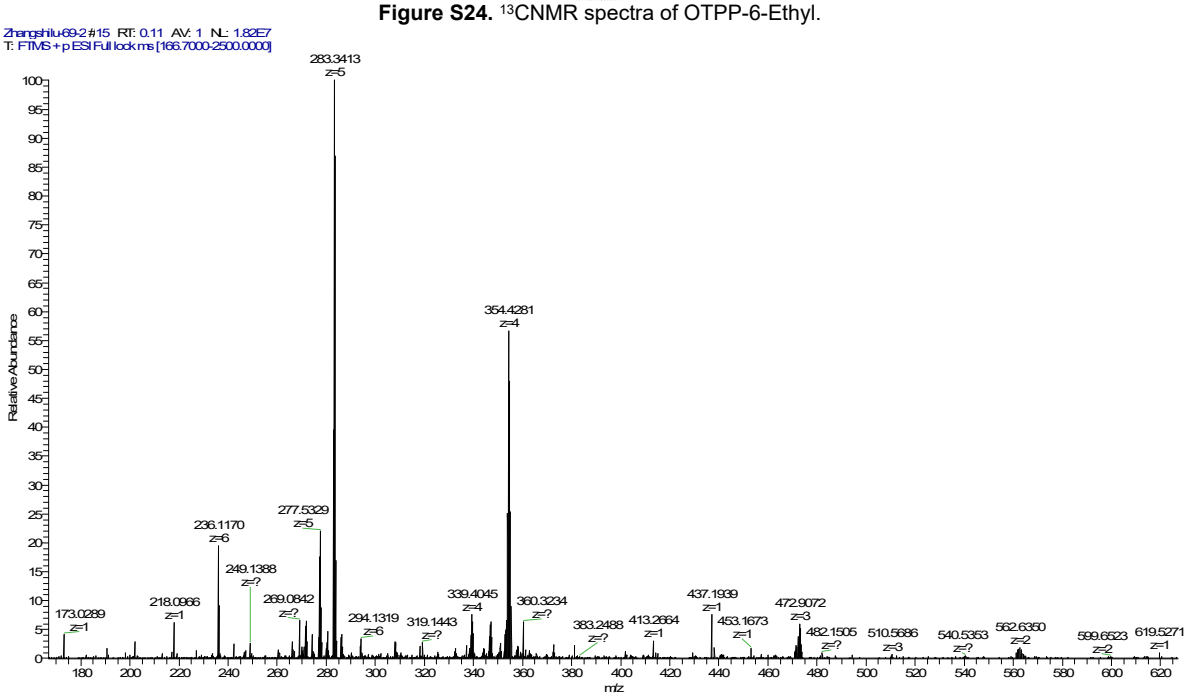


Figure S25. HRMS spectra of OTPP-6-Ethyl.

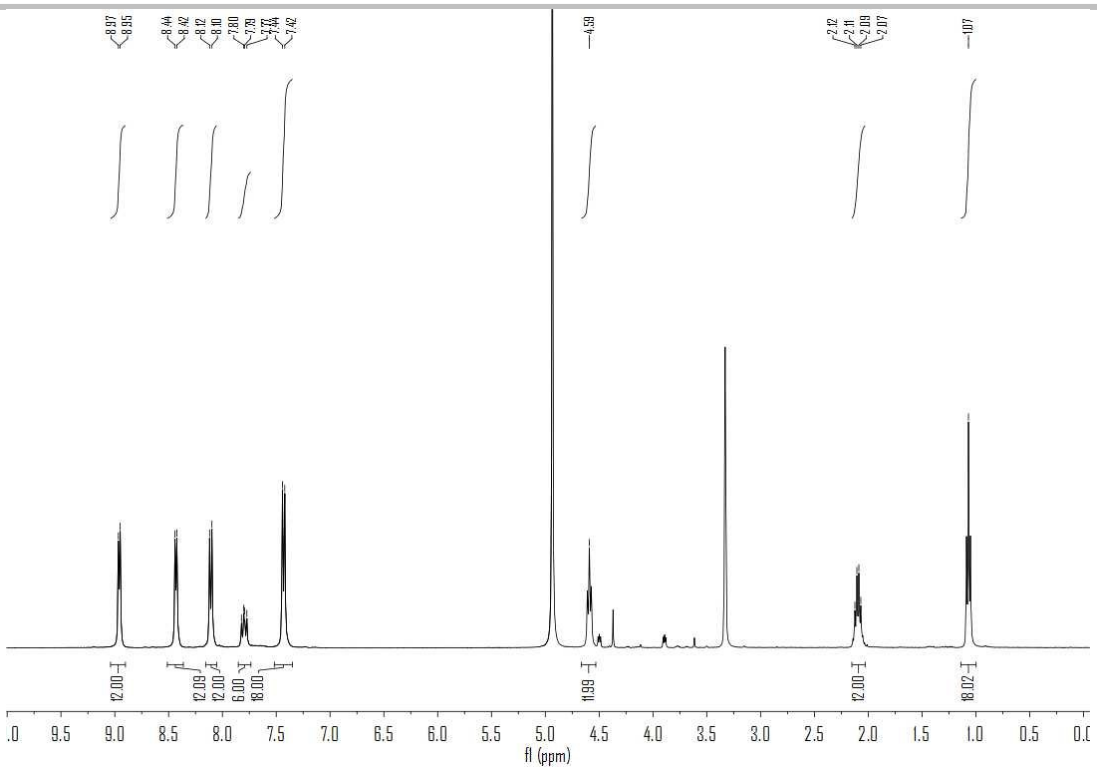


Figure S26.[1]HNMR spectra of OTPP-6-Propyl.

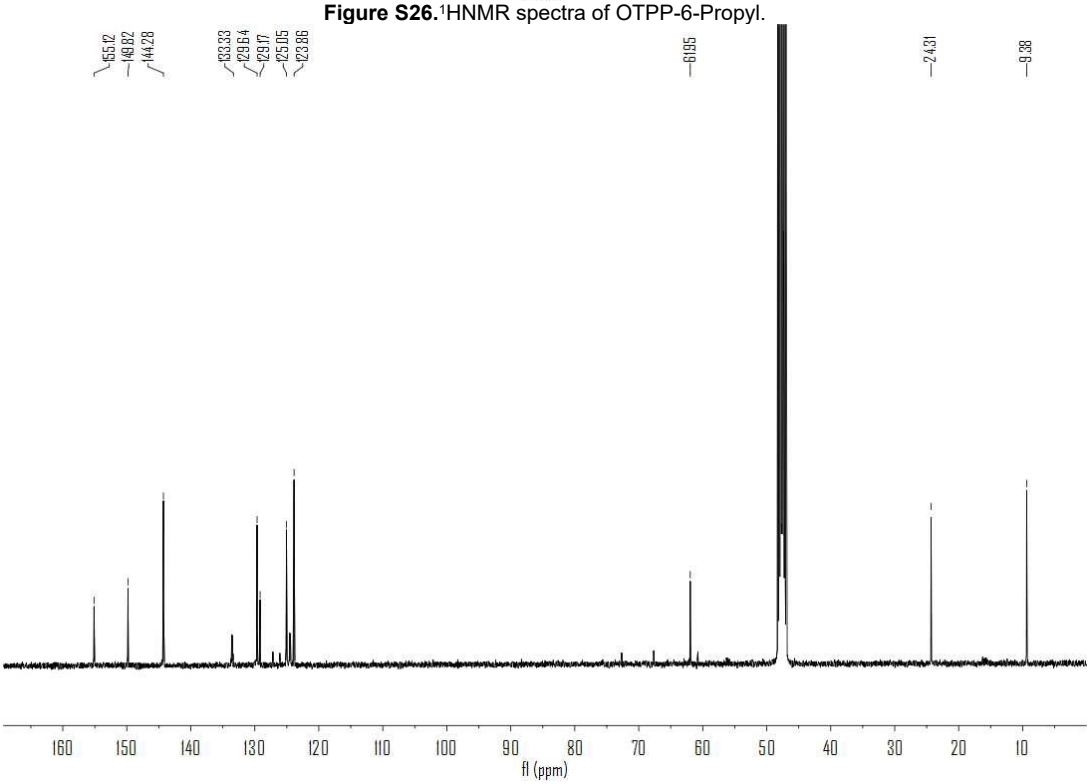


Figure S27. [13]CNMR spectra of OTPP-6-Propyl.

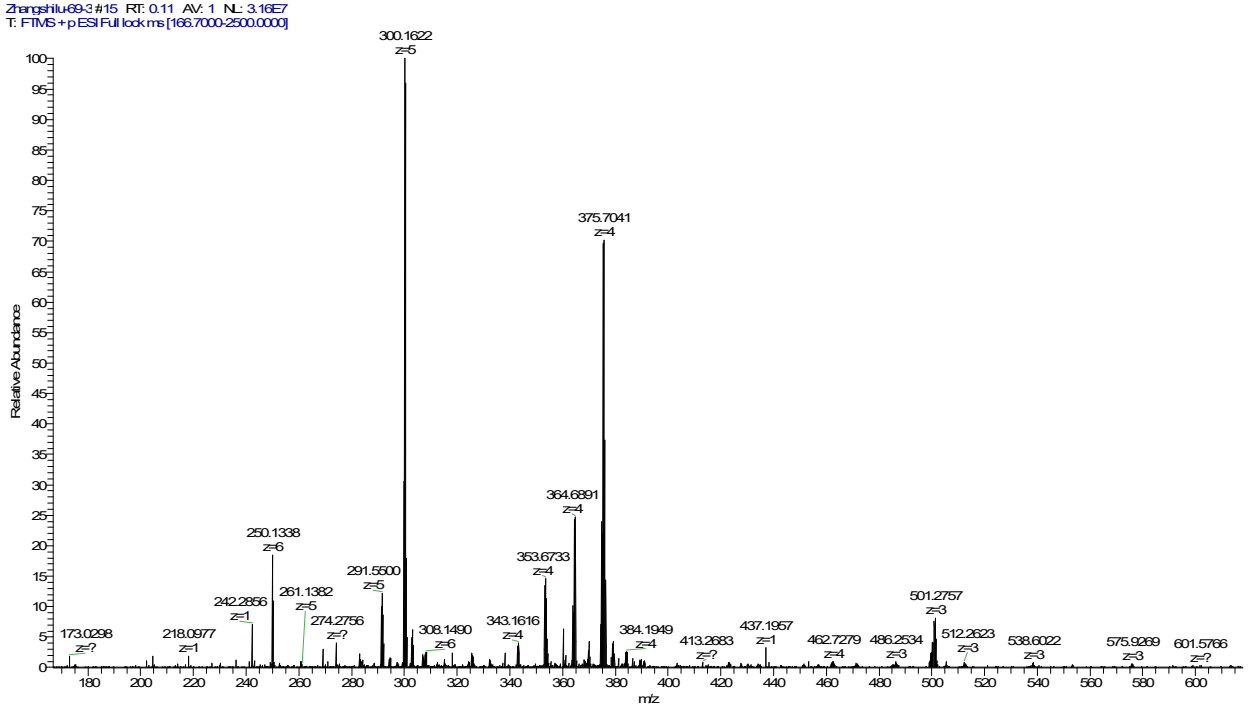


Figure S28. HRMS spectra of OTPP-6-Propyl.

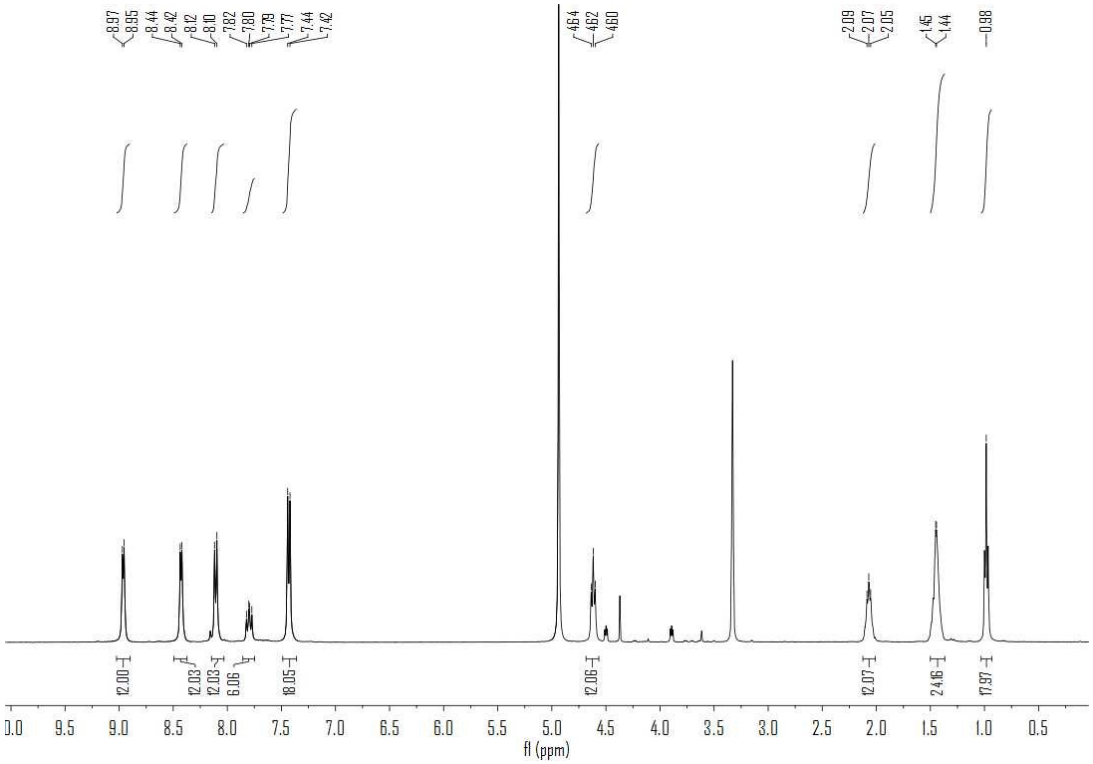


Figure S29. [1]HNMR spectra of OTPP-6-Amyl.

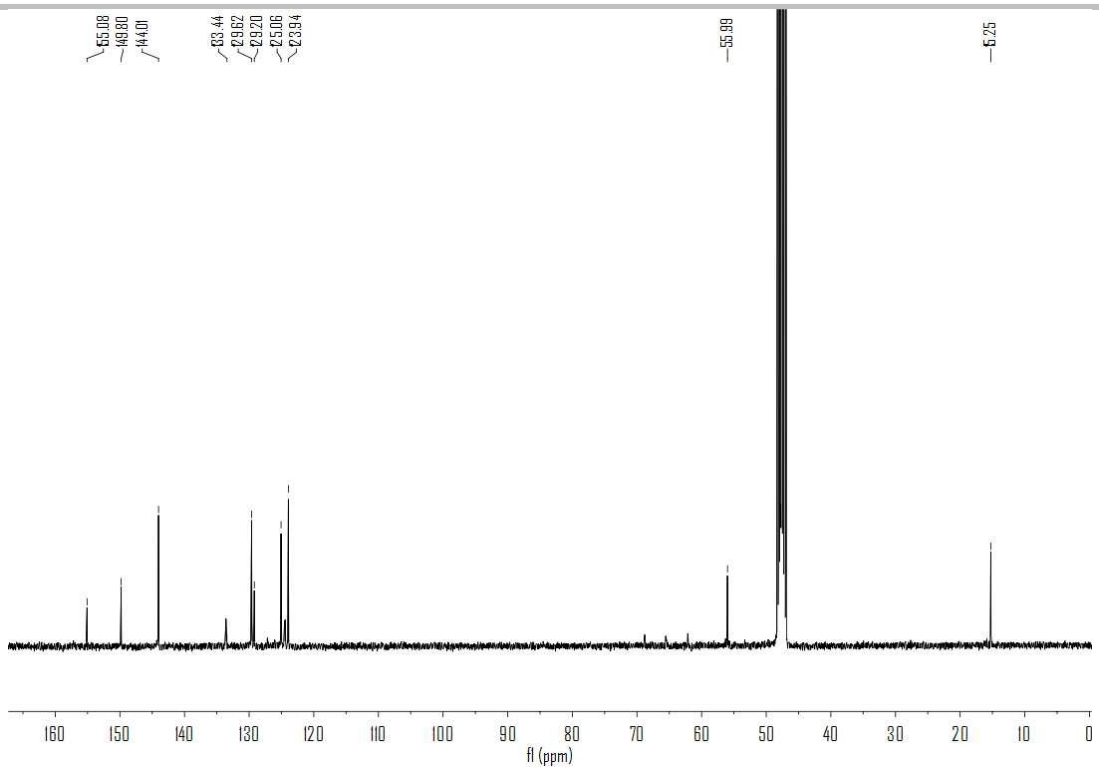


Figure S30. ^{13}CNMR spectra of OTPP-6-Amyl.

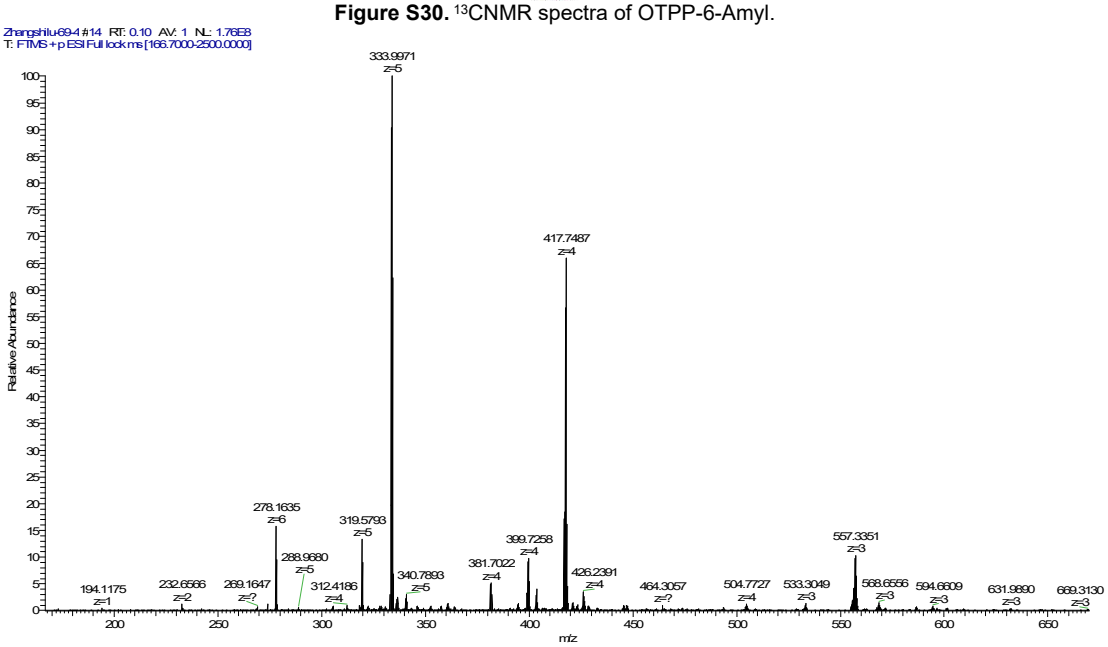


Figure S31. HRMS spectra of OTPP-6-Amyl.

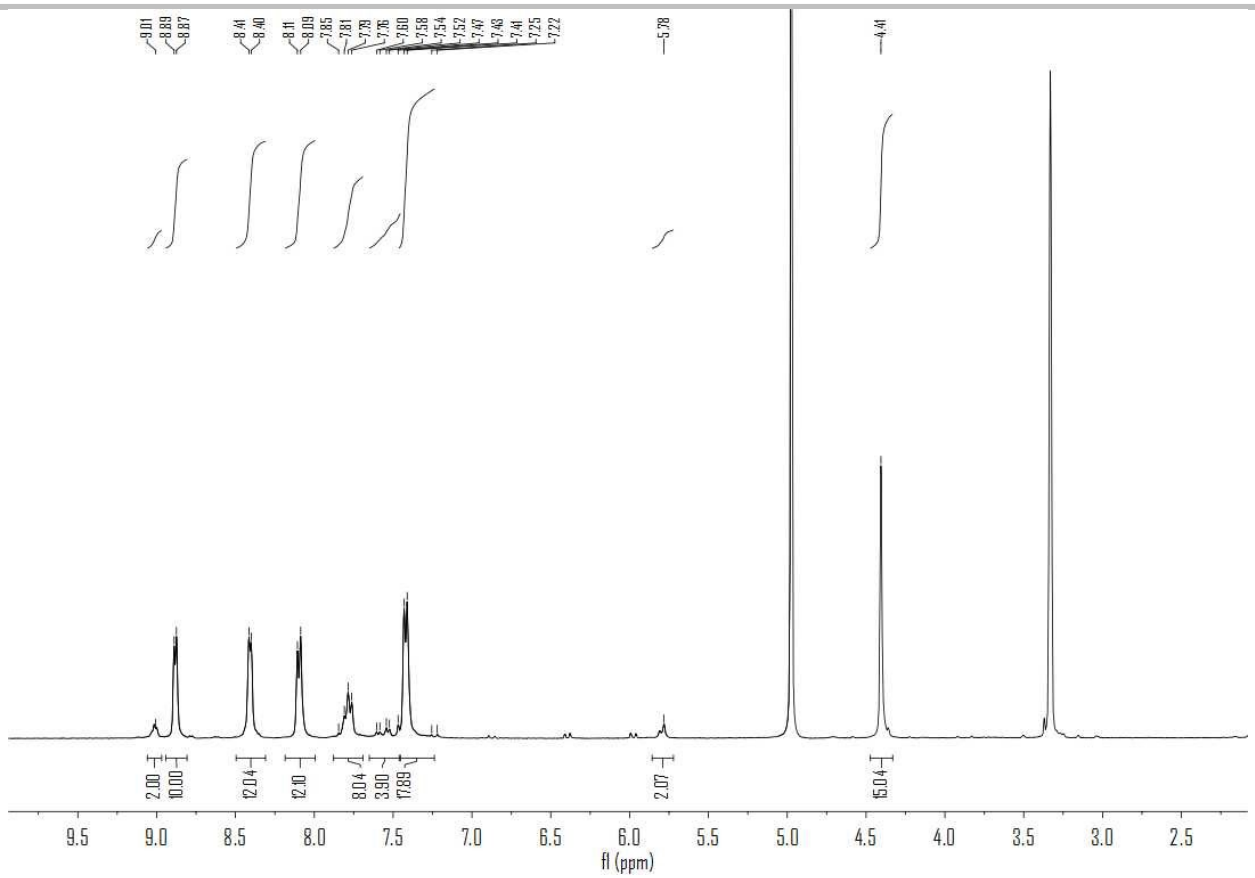


Figure S32. ^{1}HNMR spectra of OTPP-5-M-1-Mal.

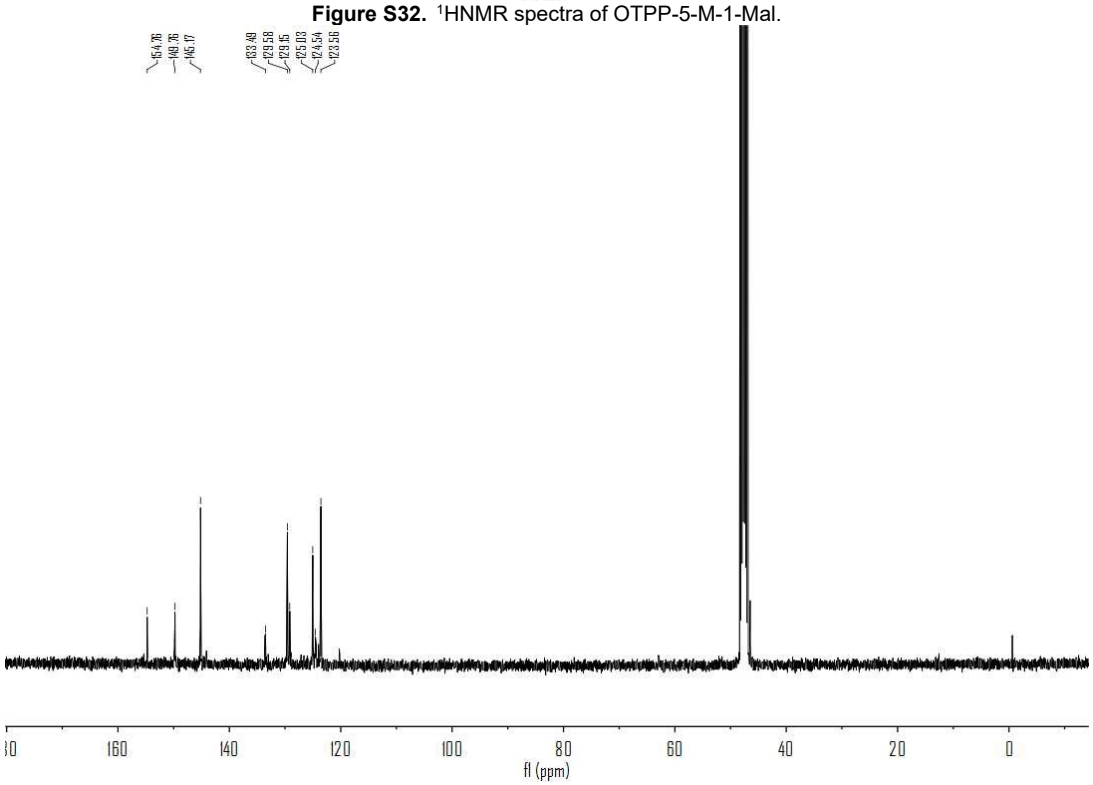


Figure S33. ^{13}CNMR spectra of OTPP-5-M-1-Mal.

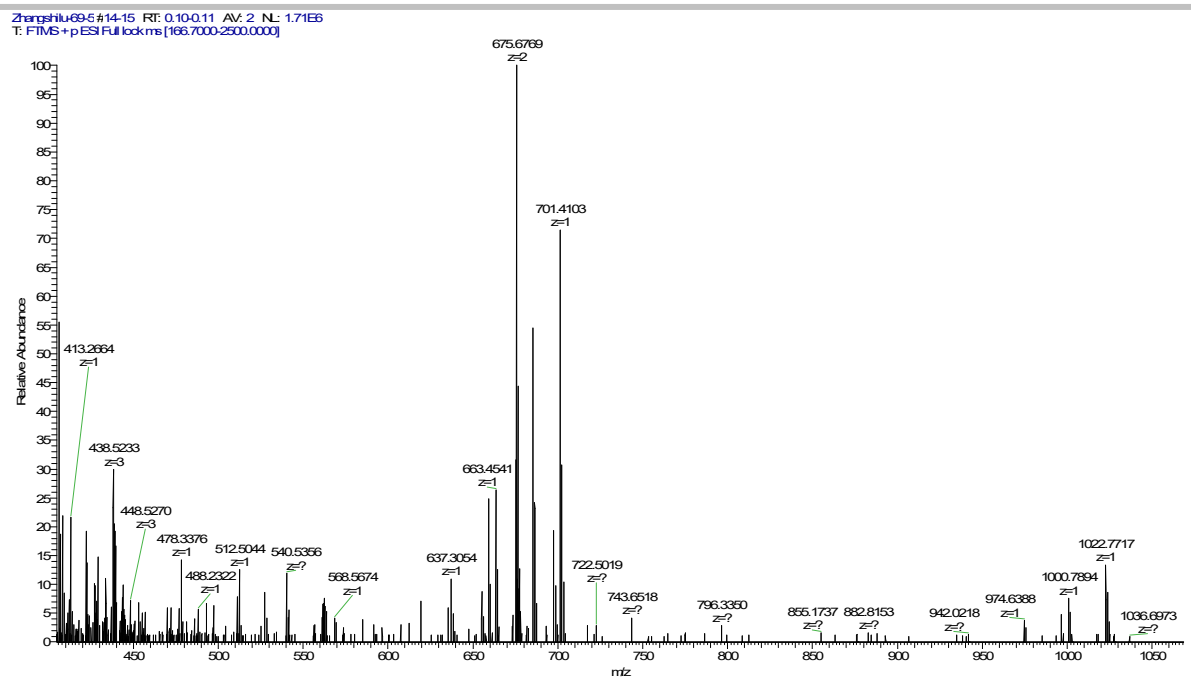


Figure S34. HRMS spectra of OTPP-5-M-1-Mal.

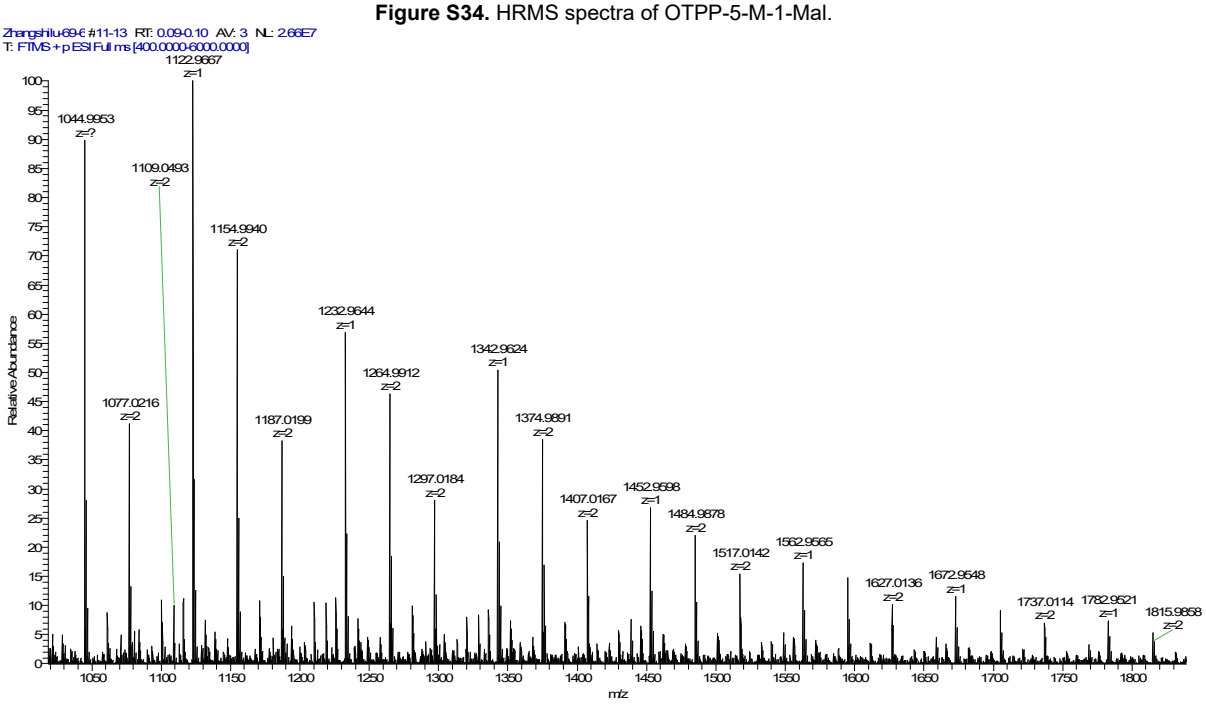


Figure S35. HRMS spectra of OTPP-5-M-1-cRGD.

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