Supporting Information

A Ratiometric Theranostic System for Visualization of $ONOO^-$ Species and Reduction of Drug-Induced Hepatotoxicity

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Materials and instrumentation

Chemicals for the synthesis were purchased from Sigma-Aldrich, J&K chemistry and Meryer as received without further purification. Bioreagents for cell culture and imaging were purchased form Thermo Fisher Scientific and Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were measured by the Bruker ARX 400 NMR spectrometer. High resolution mass spectra (HRMS) were measured by GCT premier CAB048 mass spectrometer operating in the MALDI-TOF mode. UV-Vis absorption spectra were measured on PerkinElmer Lambda 365 Spectrophotometer. Photoluminescence (PL) spectra were measured by Edinburgh FLS980 Spectrofluorometer. Particle size and distribution of AIE aggregates were measured by Zeta Potential Analyzer (ZetaPALS, Brookhaven Instruments). Fluorescent images of cells were captured by Confocal Microscopy (Zesis, LSM 800). Flow cytometry was conducted by Becton Dickinson FACS Aria IIIu Cell Sorter & Analyzer.

Generation of various RONS

The different RONS were generated according to the literature.[1]

i. $ONOO^-$: Two freshly prepared mixture solutions, including the mixture of hydrogen peroxide (0.7 M, 150 μL) and hydrochloric acid (0.6 M, 150 μL), and mixture of sodium nitrite (0.6 M, 300 μL) and sodium hydroxide (1.5 M, 300 μL), were mixed simultaneously to generate $ONOO^-$ stock solution (2 mM). The concentration of the $ONOO^-$ stock solution was determined according to the molar extinction coefficient of 1670 $M^{-1}cm^{-1}$ by measuring the absorbance at 302 nm in 0.1 M sodium hydroxide.

ii. H_2O_2: Stock solution of H_2O_2 (30%) is commercially available.

iii. •OH: •OH was generated by Fenton reaction. 20 mg of ferrous chloride was added into the solution of 10 equiv of H_2O_2. The concentration of •OH was supposed to be equal to that of Fe^{2+}.

iv. ClO^-: Sodium hypochlorite (13% active chlorine basis) is commercially available.

v. $\bullet O_2^-$: $\bullet O_2^-$ was sourced from potassium superoxide, 2 mM stock solution was prepared in DMSO.

Cell Culture and Staining

Cell culture: HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1 % penicillin-streptomycin in a 5% CO_2 humidified incubator at 37 °C.

Cell staining experiment: ATV-Py/ATV-PPB was dissolved in DMSO at stock concentration of 1 mM. The cultured HepG2 cells were seeded on a glass coverslip at density of 1.2 x 10^5 cells mL^{-1} and further cultured for 48 h. Then, the HepG2 cells were incubated with 10 μM ATV-Py for 30 min / 10 μM ATV-PPB for 8 h.

Co-staining experiment: MitoTracker Deep Red (MTDR) and BODIPY Green were dissolved in DMSO at stock concentration of 0.1 mM and 1 mM, respectively. HepG2 cells were firstly incubated with 10 μM ATV-PPB for 8 h followed by staining with 0.2 μM MTDR for 20 min. On the other hand, HepG2 cells were co-stained with 0.2 μM BODIPY Green and ATV-Py for 30 min.

Drug Treatment

Drug treatment experiment: Acetaminophen (APAP) was dissolved in DMSO at stock concentration of 2 M. HepG2 cells were pre-treated with different concentrations of APAP (1 mM, 5 mM and 10 mM) for 16 h and then co-incubated with ATV-PPB for another 8 h. Only for the time tracking experiment, HepG2 cells were incubated with 10 mM APAP and 10 μM ATV-Py at the same time.

Intracellular RONS Evaluation

Evaluation of intracellular ROS experiment: DCFH-DA was dissolved in DMSO at stock concentration of 10 mM. HepG2 cells were pre-treated with different concentration of APAP for 16 h and then co-incubated with ATV-PPB for another 8 h. After that 80 μM DCFH-DA was introduced to the cells for staining 30 min.

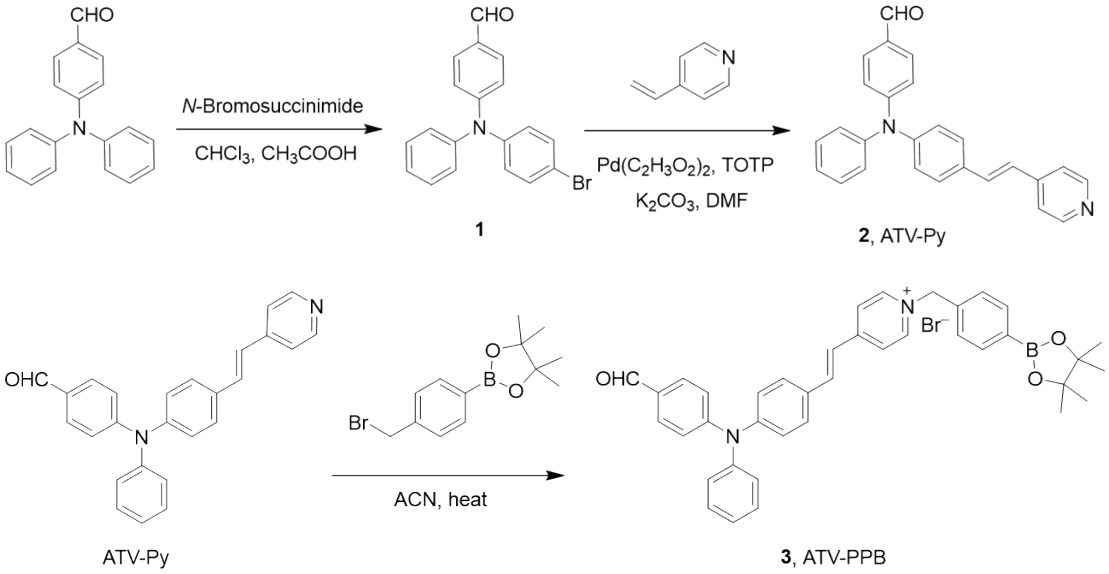
Cell Viability

HepG2 cells were seeded into 96-well plates at density of 8×10^3 to 1×10^4 cells per well and cultured for 24 h. ATV-Py and ATV-PPB were dissolved in DMEM at different concentrations (0, 1, 2, 4, 8, 10, 12, 14, 18 and 20 μM). Then, the DMEM/dye solutions were added into the wells as the treatment groups (100 μL per well) followed by 24 h incubation. After that, 10 μL freshly prepared MTT solution (5 mg mL^{-1} in PBS) was added into each well with additional 4 h incubation. Subsequently, MTT solution was carefully discarded and 100 μL DMSO was added to dissolve the purple crystals with 15 min incubation. The absorbance of MTT at 540 nm was measured by microplate reader (Varioskan LUX, Thermo Scientific). The experiment was repeated for three times.

Flow Cytometry

HepG2 cells were seeded in 6-well plates at density of 4×10^4 cells per well and cultured for 48 h. HepG2 cells were pre-treated with different concentrations of APAP (1 mM, 5 mM and 10 mM) for 16 h and then co-incubated with ATV-PPB for another 8 h., HepG2 cells were pre-treated with different concentrations of APAP (1 mM, 5 mM and 10 mM) for 24 h and 10 μM ATV-PPB was added at 16 h for 8 h staining. The fluorescence signals were measured by flow cytometry and collected from DAPI and PerCP-Cy5-5 channel.

Synthesis of AIEgens



Scheme S1. The synthetic route of ATV-Py and ATV-PPB.

Synthesis of 4-((4-bromophrnyl)(phenyl)amino)benzaldehyde (1): Into a 20 mL one-neck round-bottom flask, 4-(diphenylamino)benzaldehyde (2.71 g, 10 mmol) and *N*-bromosuccinimide (1.775 g, 10 mmol) were dissolved in chloroform(5 mL) with acetic acid (5 mL). The mixture was then stirred in room temperature. After 24 h reaction, the reaction mixture was extracted with DCM and H_2O, organic layer was collected and dried over by $MgSO_4$ and concentrated by evaporation. The crude product was purified by silica gel chromatography with hexane/ethyl acetate (4:1 v/v) to obtain **1** as white powder (2.04 g, 57.9 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (*s*, 1H), 7.72–7.66 (*m*, 2H), 7.45–7.41(*m*, 2H), 7.36–7.32 (*m*, 2H), 7.20–7.14 (*m*, 3H), 7.05–7.02 (*m*, 4H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 190.5, 152.9, 145.9, 145.4, 133.0, 132.9, 131.5, 131.4, 130.0, 129.8, 129.7, 127.5, 126.4, 126.3, 125.5, 125.2, 120.5, 120.0, 119.4, 117.8. HRMS (MALDI-TOF, *m*/*z*): $[M]^+$ calcd for $C_{19}H_{14}BrNO$ 352.2310, found 352.0323.

Synthesis of (*E*)-4-(phenyl(4-(2-(pyridine-4-yl)vinyl)phenyl)amino)benzaldehyde (2 or ATV-Py): Into a 50 mL two-neck round-bottom flask, **1** (1.098 g, 3 mmol), 4-vinylpyridine (0.644 mL, 6 mmol), palladium(II) acetate (0.067 g, 0.3 mmol), tris-o-tolyphosphine (0.2739 g, 0.9 mmol) and potassium carbonate (2.902 g, 21 mmol) were dissolved in DMF (20 mL) under N_2. The mixture was heated to 150 °C and stirred overnight. After 24 h reaction, the reaction mixture was cooled down to room temperature and extracted with extracted with DCM for three times. All the organic layers were collected and dried over by $MgSO_4$ and concentrated by evaporation. The crude product was purified by silica gel chromatography with hexane/ethyl acetate (1:1 v/v) to obtain ATV-Py (**2**) as yellow powder (0.785 g, 67.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (*s*, 1H), 8.59 (*s*, 2H), 7.72 (*d*, 2H), 7.50 (*d*, 2H), 7.40–7.36 (m, 4H), 7.33–7.27 (*m*, 1H), 7.20 (*m*, 5H), 7.10 (*d*, 2H), 6.99–6.95 (*m*, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 190.5, 152.9, 150.3, 146.7, 146.0, 144.7, 132.5, 132.3, 131.4, 130.0, 129.9, 128.4, 126.5, 125.6, 125.5, 120.5. HRMS (MALDI-TOF, *m*/*z*): $[M]^+$ calcd for $C_{26}H_{20}N_2O$ 376.1580, found 377.1653.

Synthesis of (*E*)-4-(4-((4-formylphenyl)(phenyl)amino)styryl)-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyridine-1-ium bromide (3 or ATV-PPB): Into a 20 mL one-neck round-bottom flask **2** (0.391 g, 1 mmol) and 4-bromomethylphenylboronic acid pinacol ester (0.297 g, 1 mmol) were dissolved in acetonitrile (10 mL). The mixture was heated to 100 °C and stirred. After 24 h reaction, the reaction mixture was cooled down to room temperature and concentrated. The crude product was purified by silica gel chromatography with ethyl acetate/methanol (5:1 v/v) to obtain ATV-PPB (**3**) as red powder (0.368 g, 62.0 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), 9.07–9.06 (*m*, *2*H), 7.94 (*d*, 2H), 7.77 (*d*, 2H), 7.72–7.70 (*m*, 2H), 7.65 (*m*, 1H), 7.57–7.55 (*m*, 2H), 7.52–7.50 (*m*, 1H), 7.36–7.32 (*m*, 2H), 7.22–7.18 (*m*, 1H), 7.13–7.11 (*m*, 2H), 7.11–7.10 (*m*, 3H), 7.10– 7.08 (*m*, 1H), 7.01 (*m*, 1H), 6.01 (*s*, 2H), 1.29 (*s*, 12H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 190.7, 152.4, 145.7, 144.1, 141.6, 136.0, 131.4, 130.7, 130.2, 130.0, 128.6, 126.9, 126.1, 124.3, 123.9, 121.8, 121.0, 84.2, 63.4, 25.0. HRMS (MALDI-TOF, *m*/*z*): $[M]^+$ calcd for $C_{39}H_{38}BN_2O_3$ 593.2980, found 593.2973.

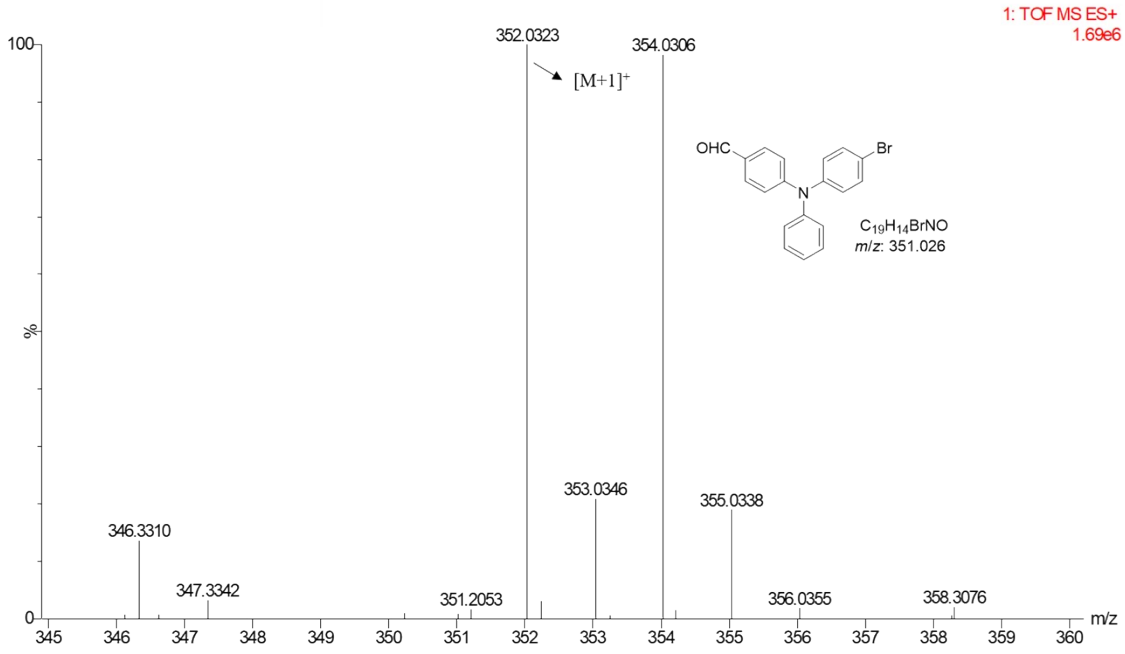


Figure S1. Mass spectrum of **1**.

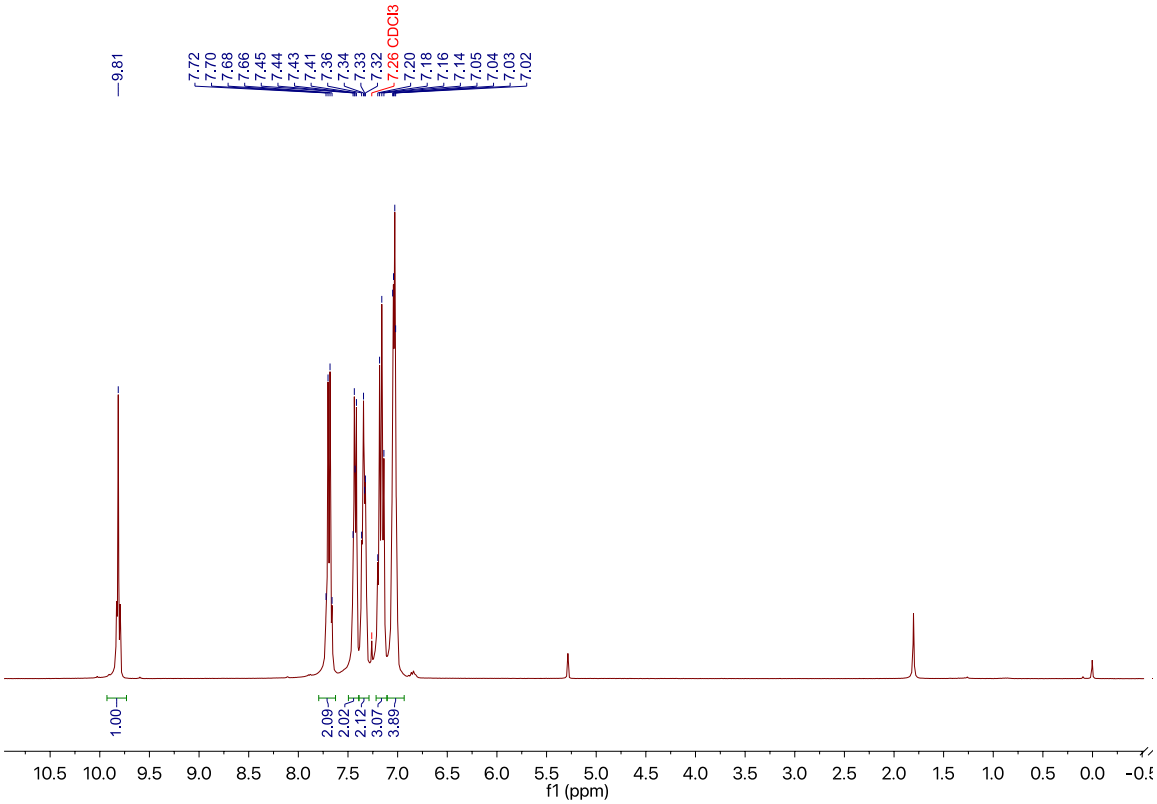


Figure S2. ^{1}H NMR spectrum of **1** in $CDCl_3$.

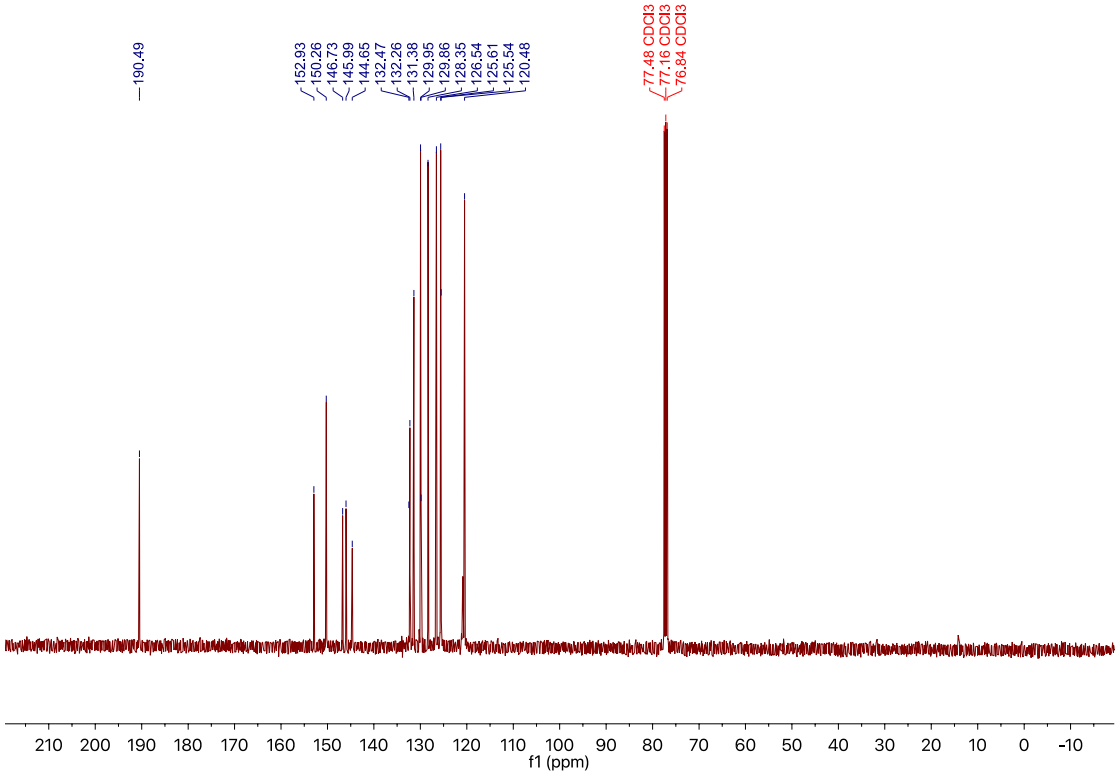


Figure S3. ^{13}C NMR spectrum of **1** in $CDCl_3$.

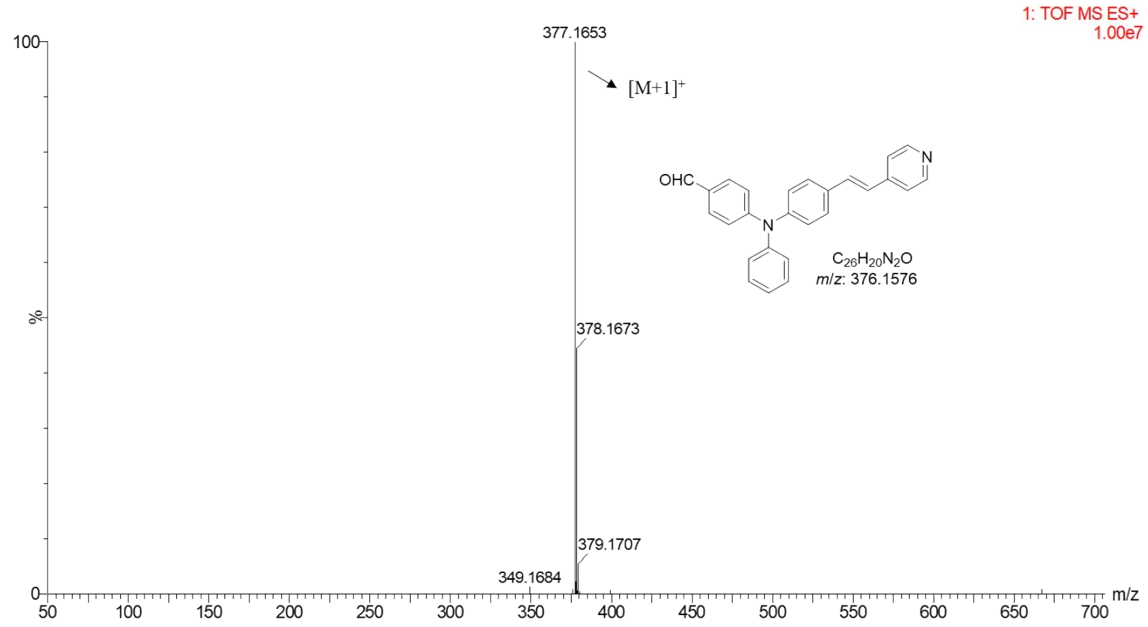


Figure S4. Mass spectrum of ATV-Py (**2**).

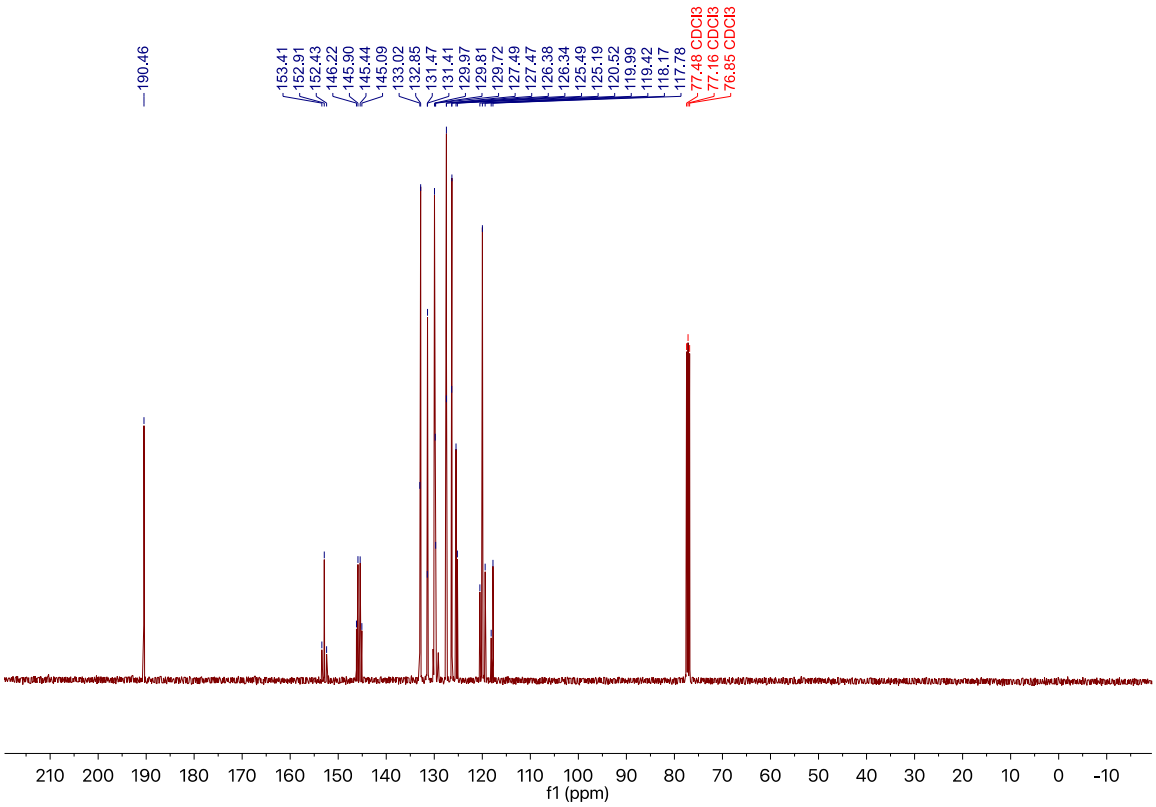


Figure S5. ^{1}H NMR spectrum of ATV-Py (**2**) in $CDCl_3$.

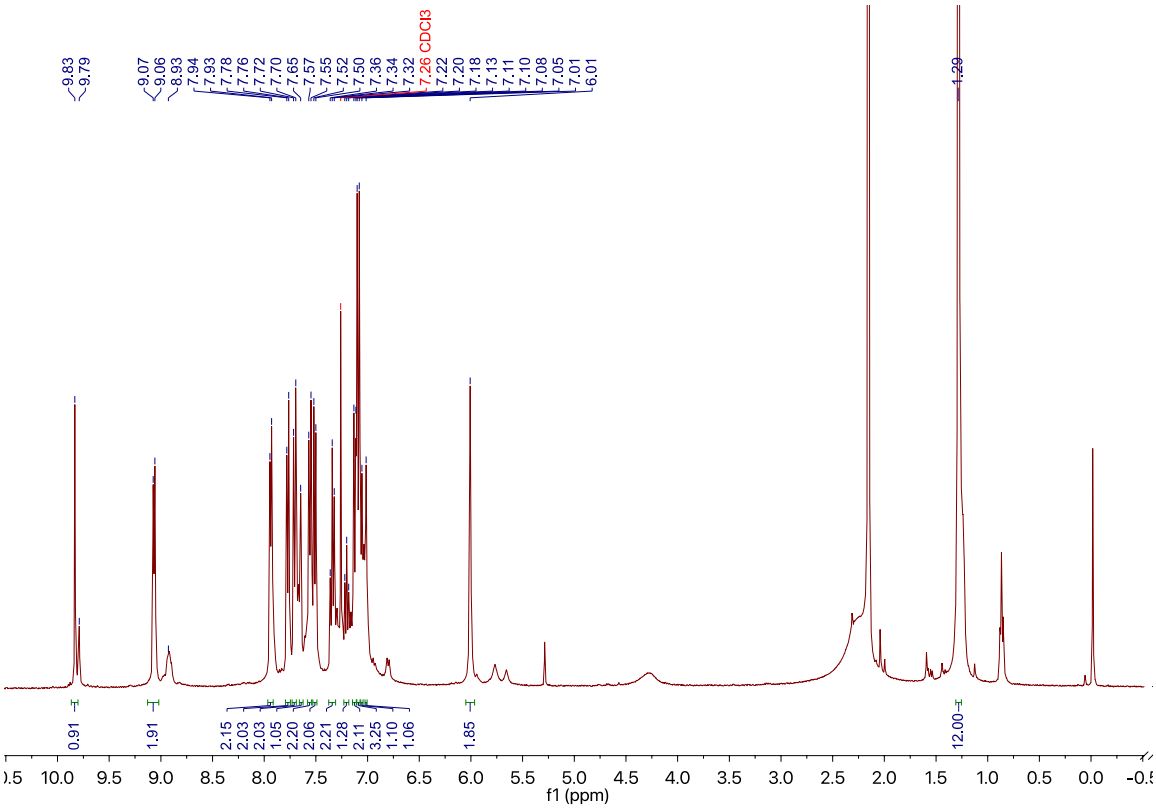


Figure S6. ^{13}C NMR spectrum of ATV-Py (**2**) in $CDCl_3$.

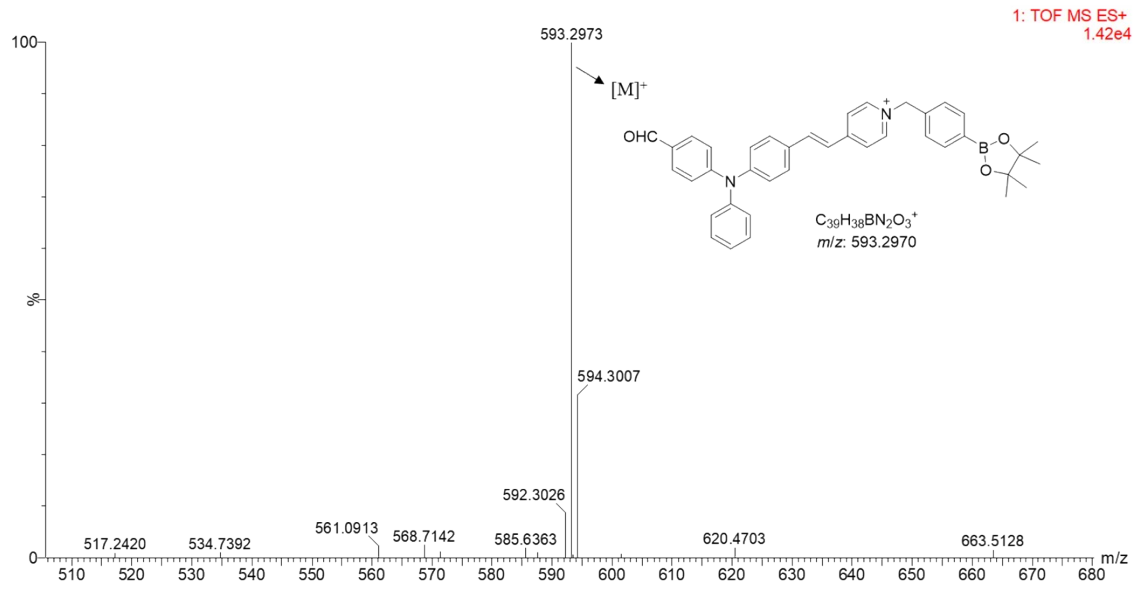


Figure S7. Mass spectrum of ATV-PPB (**3**).

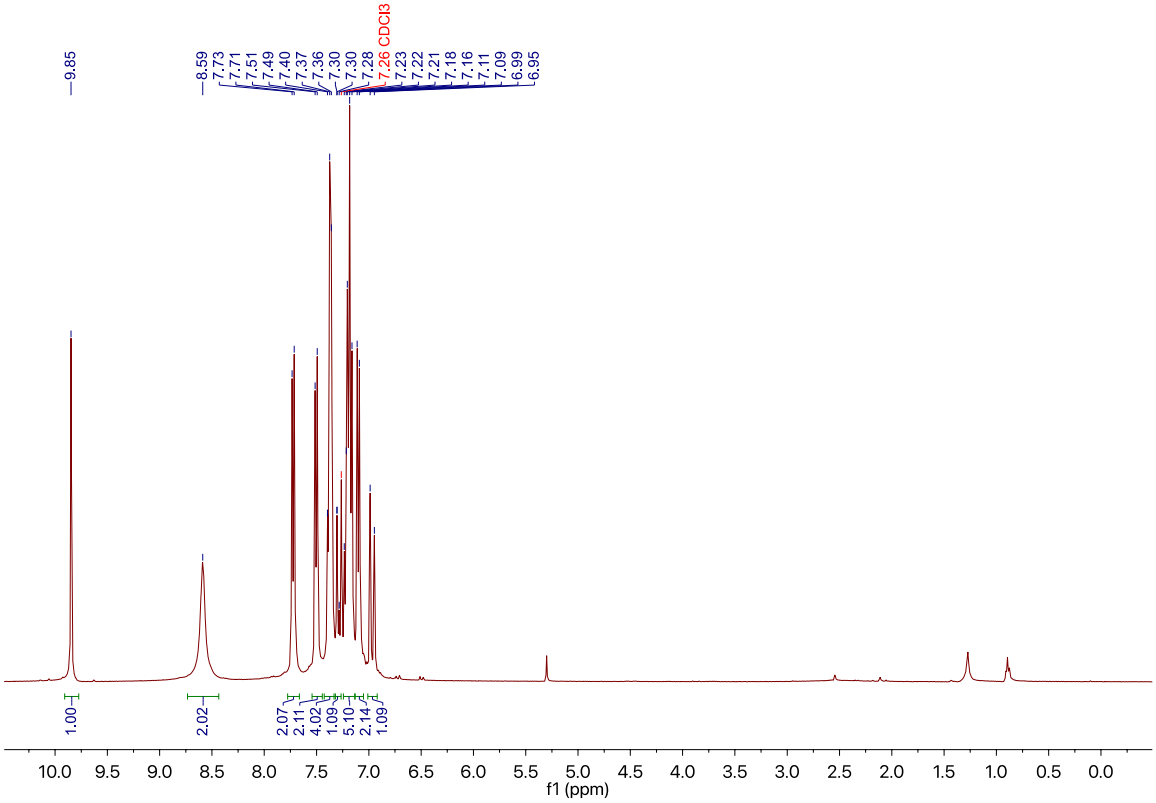


Figure S8. ^{1}H NMR spectrum of ATV-PPB (**3**) in $CDCl_3$.

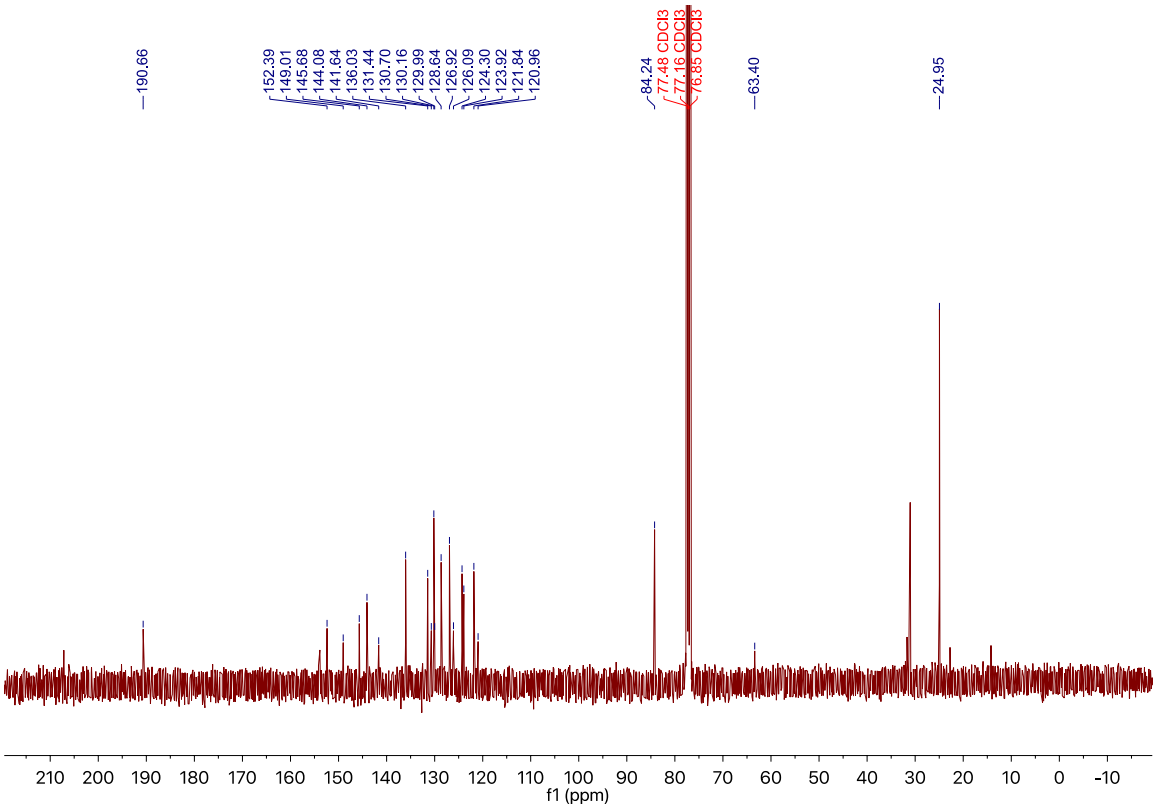


Figure S9. ^{13}C NMR spectrum of ATV-PPB (**3**) in $CDCl_3$.

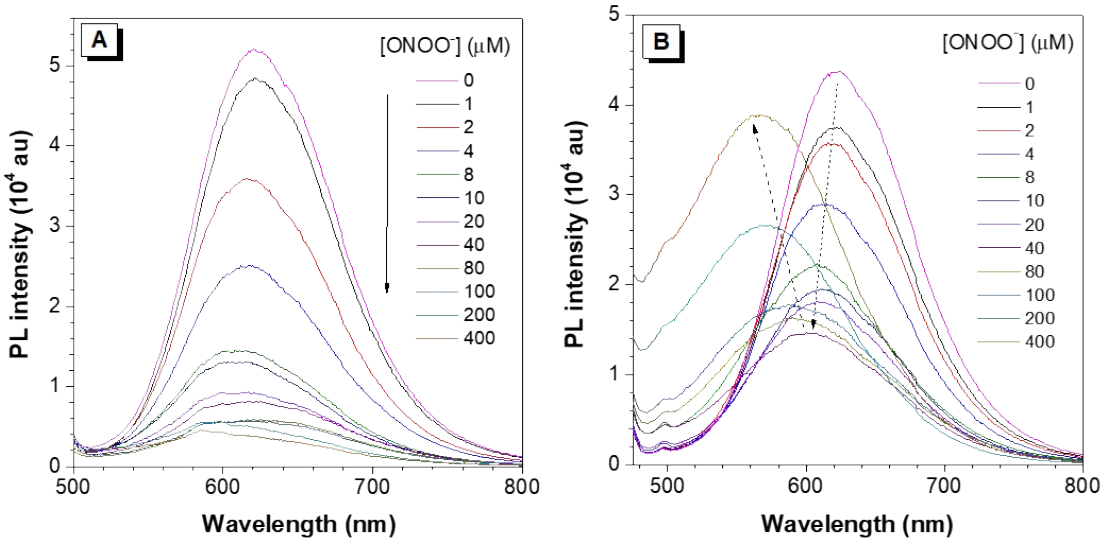


Figure S10. PL spectra of 10 μM ATV-PPB in PBS buffer with different concentrations of $ONOO^-$ under light excitation of (A) 488 nm and (B) 405 nm.

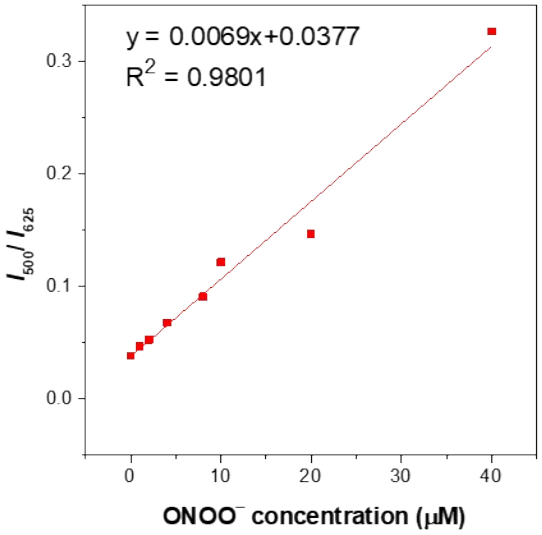


Figure S11. Plot of PL intensity ratio (I_{500}/ I_{625}) as a function of $ONOO^-$ concentration.

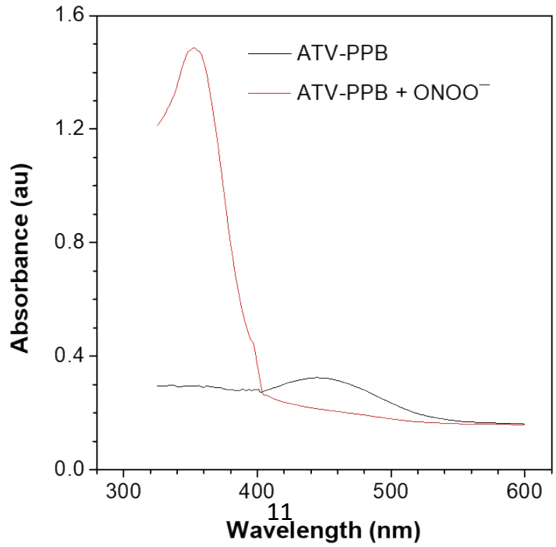


Figure S12. Absorption spectra of 10 μM ATV-PPB (red) with or (black) without addition of 400 μM $ONOO^-$ in PBS buffer.

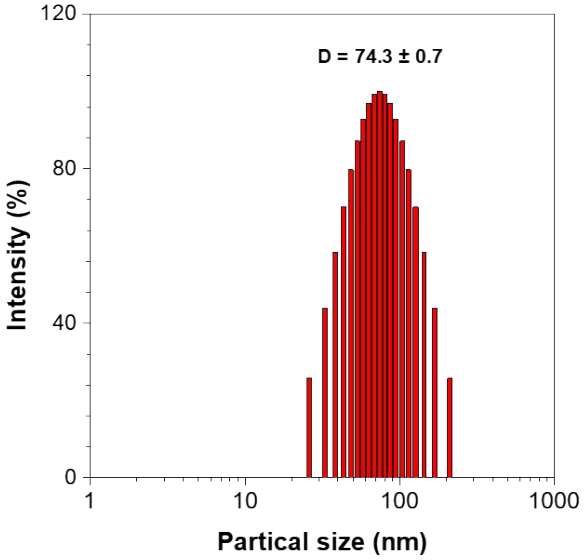


Figure S13. Hydrodynamic size distribution of ATV-PPB in 10% DMSO of DMSO/H_2O mixture. D represented as mean ± S.D. (n = 6).

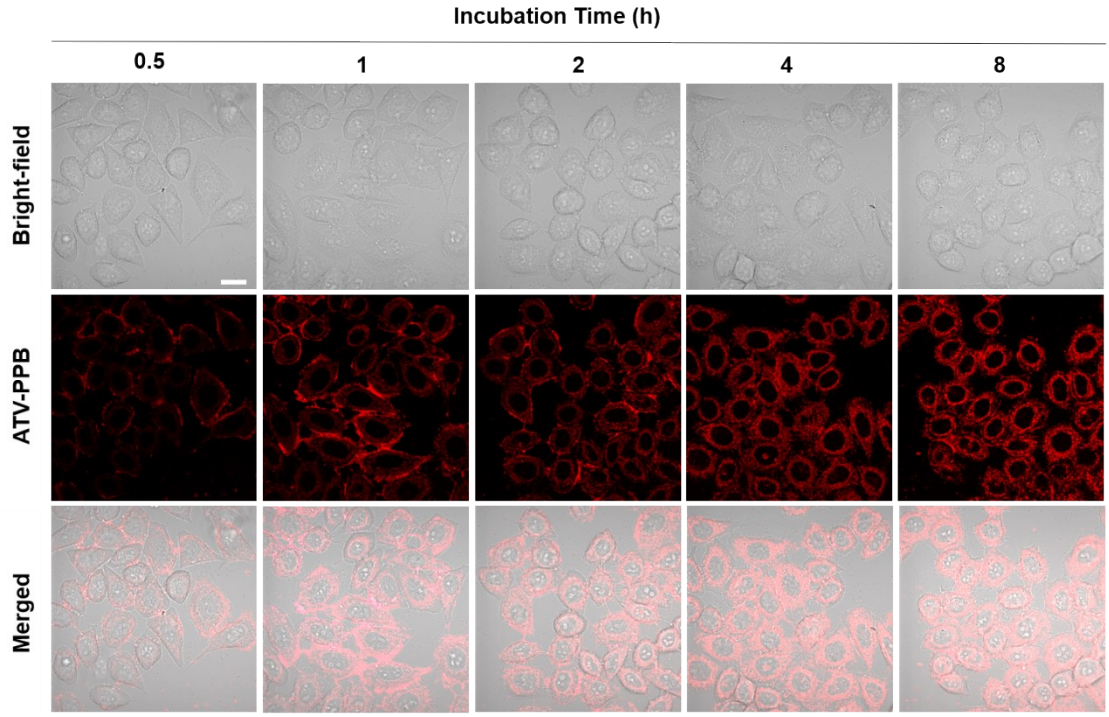


Figure S14. Time tracking of ATV-PPB-stained HepG2 cells from 0.5 h to 8 h. ATV-PPB concentration =10 mM; λ_{ex}= 488 nm; λ_{em}= 600-700 nm. Scale bar: 20 μm.

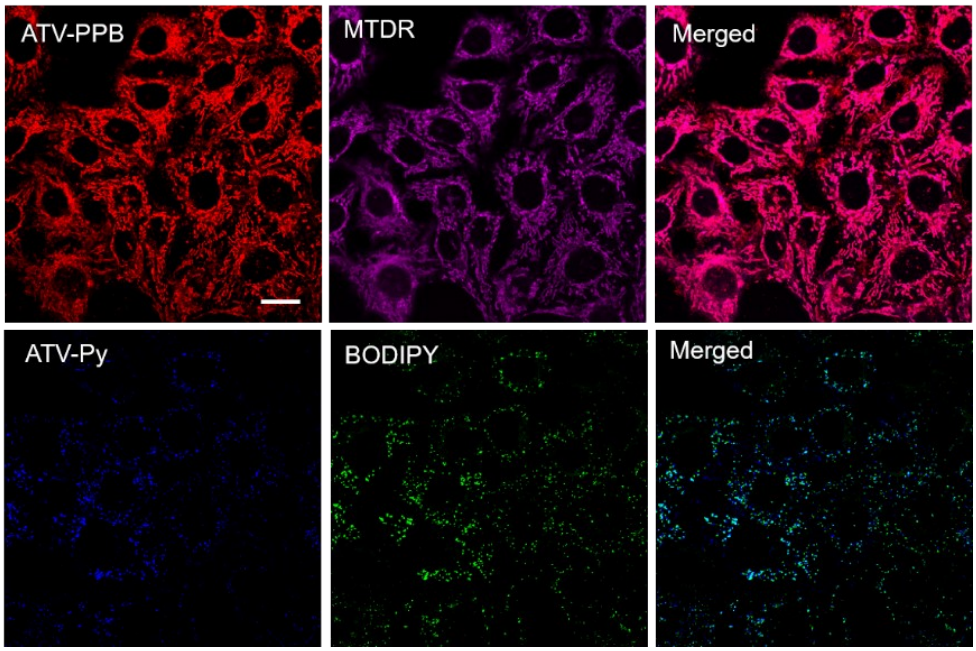


Figure S15. CLSM images of HepG2 cells were first treated with 10 mM APAP for 16 h and then stained with 10 μM ATV-PPB for another 8 h followed by staining with 0.2 μM MTDR/ 0.2 μM BODIPY493/503 Green for next 30 min. ATV-PPB: λ_{ex}= 488 nm, λ_{em}= 600-700 nm; ATV-Py: λ_{ex}= 405 nm, λ_{em}= 450-600 nm; MTDR: λ_{ex}= 561 nm, λ_{em}= 600-700 nm; BODIPY493/503 Green: λ_{ex}= 488 nm, λ_{em}= 500-550 nm. Scale bar: 20 μm.

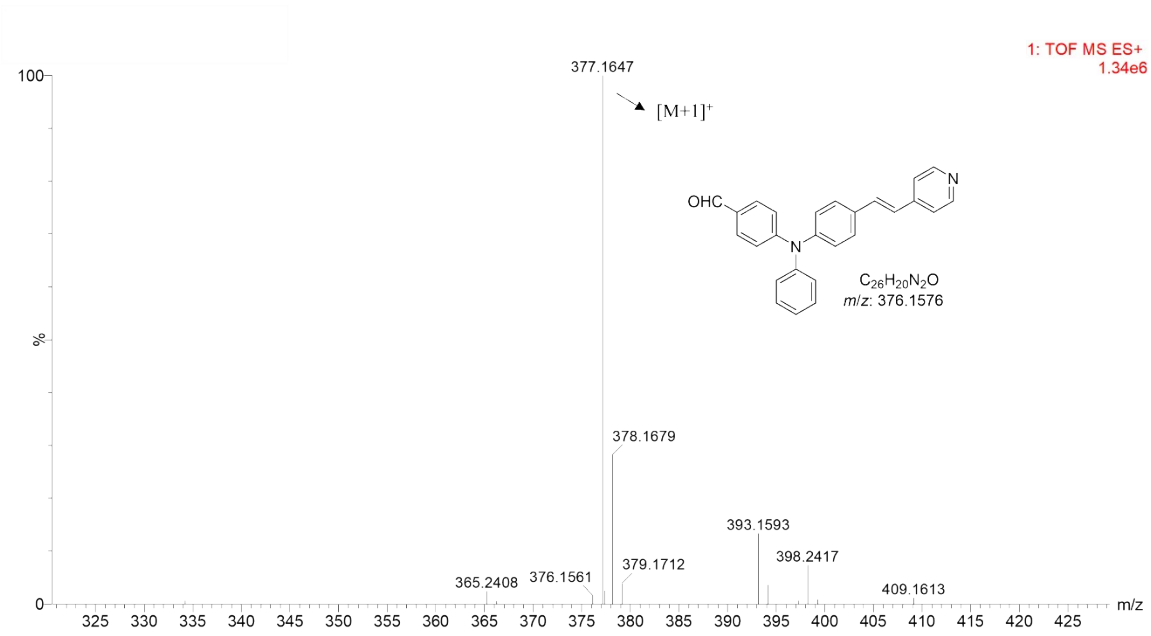


Figure S16. Mass spectrum of the reaction product (10 μM ATV-PPB and 400 μM $ONOO^-$ reacted for 30 min).