Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

A Fluorescent Probe Strategy for the Detection and Discrimination of Hydrogen Peroxide and Peroxynitrite in Cells

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Table of Contents

1. General Information and Methods

Materials and Chemicals

All chemicals and reagents were purchased commercially and were of analytical grade. Absorption spectra were obtained on a Jasco V-770 spectrophotometer and fluorescence spectra were obtained on a PerkinElmer LS55 Luminscence spectrometer using quartz cuvettes of 1 cm path length. Column chromatography was carried out on Merck® silica gel 60 under a positive pressure of nitrogen. Eluent ratios are reported by volume percentages. NMR spectra were recorded on a Bruker AVIII 400, Bruker NEO 600, Bruker AVII 500 (with cryoprobe) and Bruker AVIII 500 spectrometers. Chemical shifts are reported as δ values in ppm. Mass spectra were carried out on a Waters Micromass LCT and Bruker microTOF spectrometers. Peroxy Orange 1 was purchased from Tocris, Bio-Techne, this was dissolved in DMSO (Sigma) to make a 10 mM stock solution, this was kept at -20°C.

Cell Culture

A549, H460 cells (lung adenocarcinoma) and HCT116 (colorectal carcinoma) were obtained from ATCC and maintained in DMEM media (Sigma) supplemented with 10% FBS (Sigma). Cells were cultured in a humidified incubator at 37°C and 5% CO_2. Cell lines were routinely tested for mycoplasma and found to be negative.

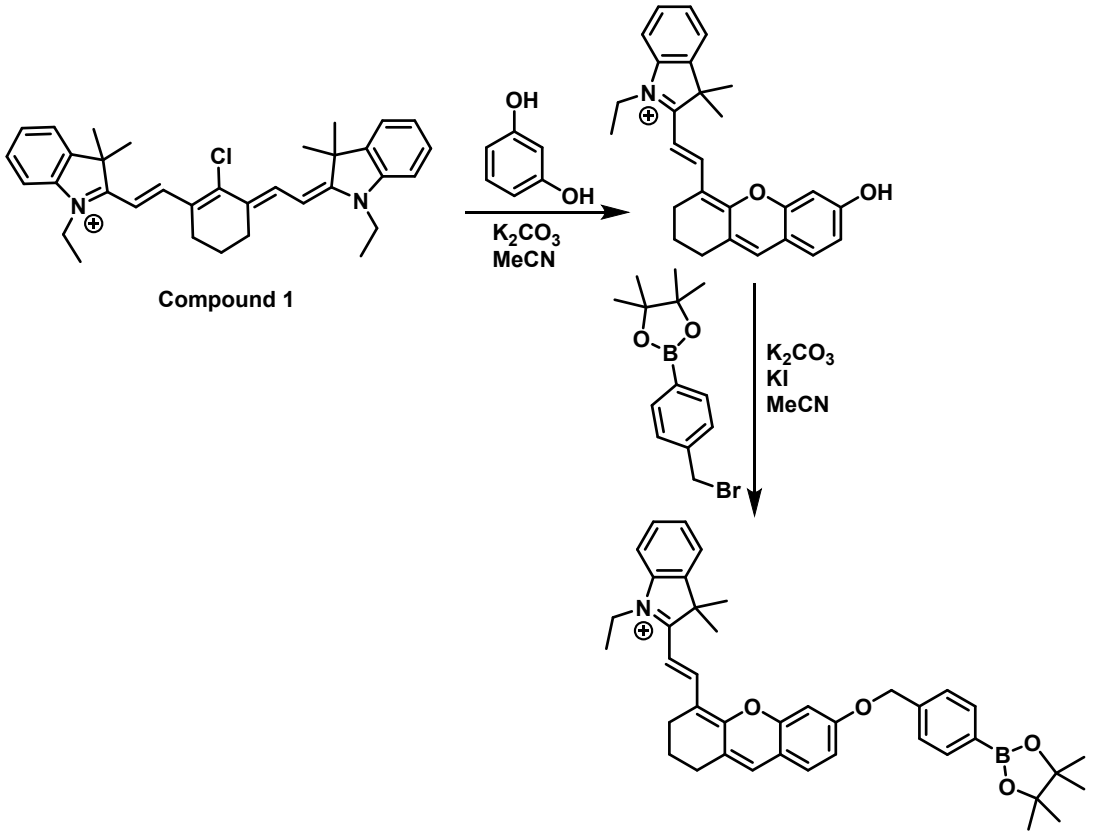
MTT Assay

5,000 cells per well were seeded and allowed to adhere overnight. Cells were treated with either 10, 20 or 40 μM of HD-BPin for 30 minutes. **HD-BPi**n was removed, and fresh media was added. MTT assay was then carried out 24 hours later. Cells were incubated with 0.5 mg/mL MTT reagent (Sigma) in complete media for 3 hours at 37°C protected from light. MTT was removed and formazan crystals were solubilized with 100 μl of DMSO for 15 minutes at 37°C protected from light. Absorbance was read at 570 nm (Clariostar, BMG). Data expressed as percentage viability relative to untreated control.

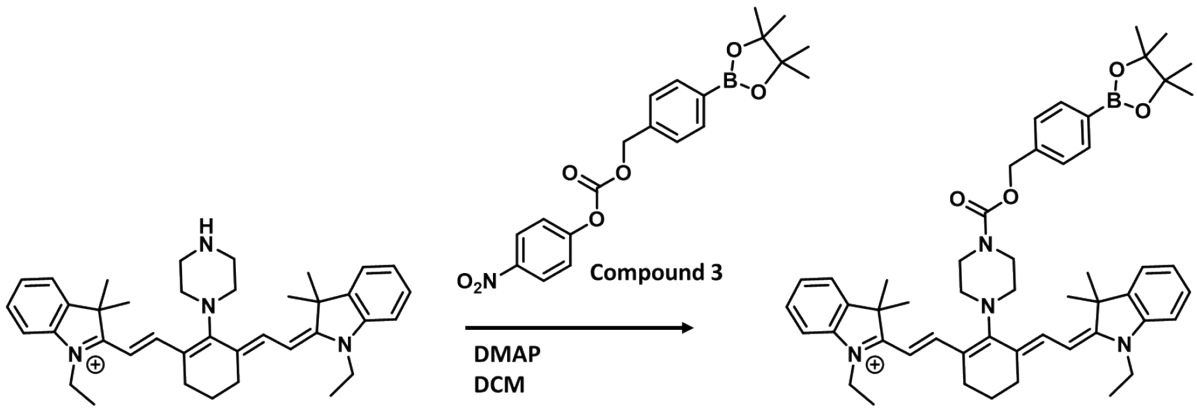
Fluorescent Microscopy and Quantification

Cells were seeded onto glass coverslips (Menzel-Glaser) before treatment. Cells were treated with HD-BPin, donors, scavengers and Cisplatin. Cells were fixed in 4% PFA (Sigma) for 10 minutes. Coverslips were mounted onto microscope slides using ProLong™ Gold Anti Fade Mountant without DAPI (Thermo Fisher). Cells were imaged with a LSM780 confocal microscope (Carl Zeiss Microscopy Ltd). At least 100 cells per condition were counted. Fluorescent intensity per cell was calculated using a custom script generated from CellProfiler cell image analysis software.

2. Synthetic Schemes



Scheme S1. Synthesis of **HD-BPin**



Scheme S2. Synthesis of **Cy7-BPin**

3. Additional Analyses

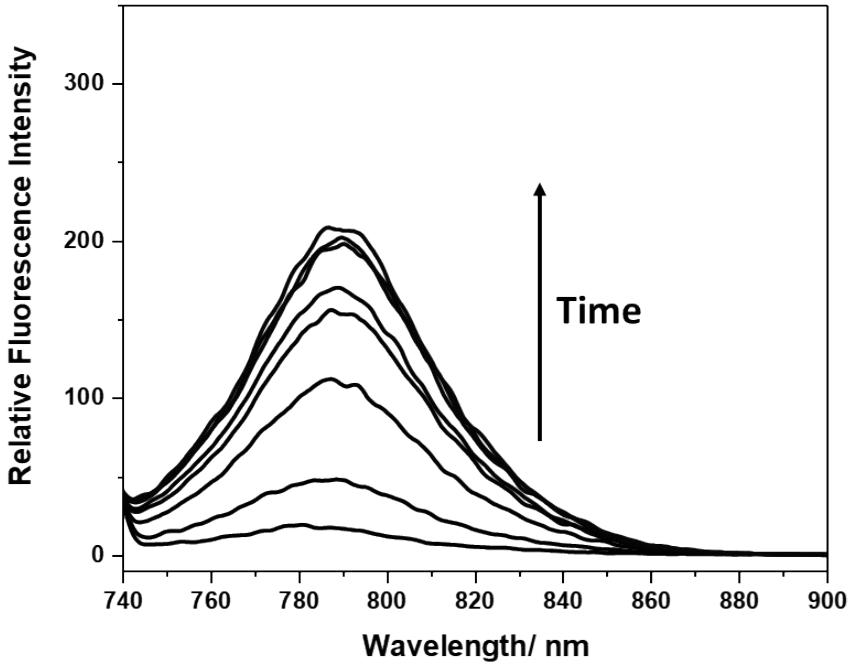


Figure S1. Fluorescence spectra of **Cy7-BPin** (10 μM) in PBS (pH = 7.20) and H_2O_2 (10 mM). Measurements were recorded every 5 mins, λ_{ex} = 730 nm (Slit widths: 10 nm and 10 nm).

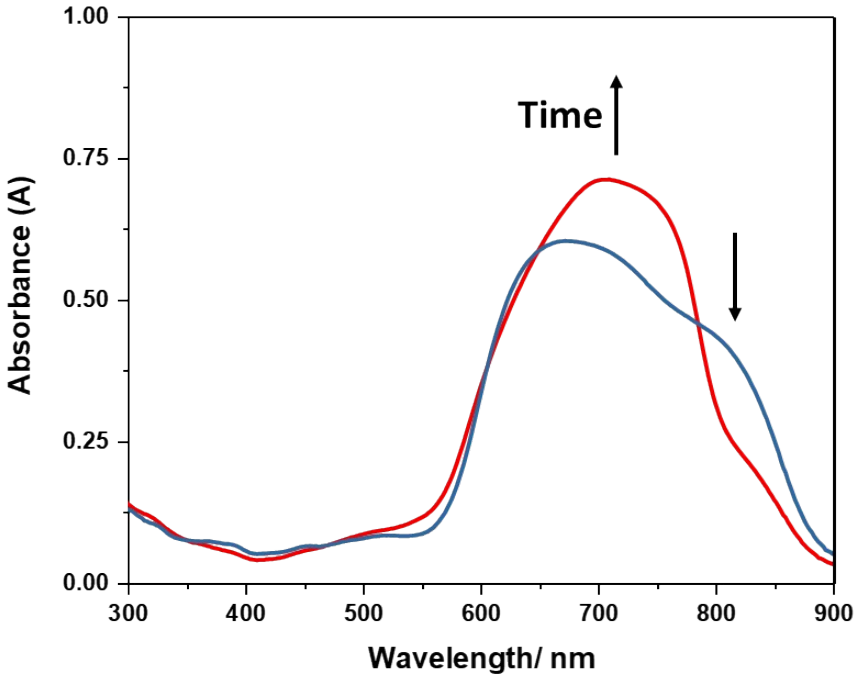
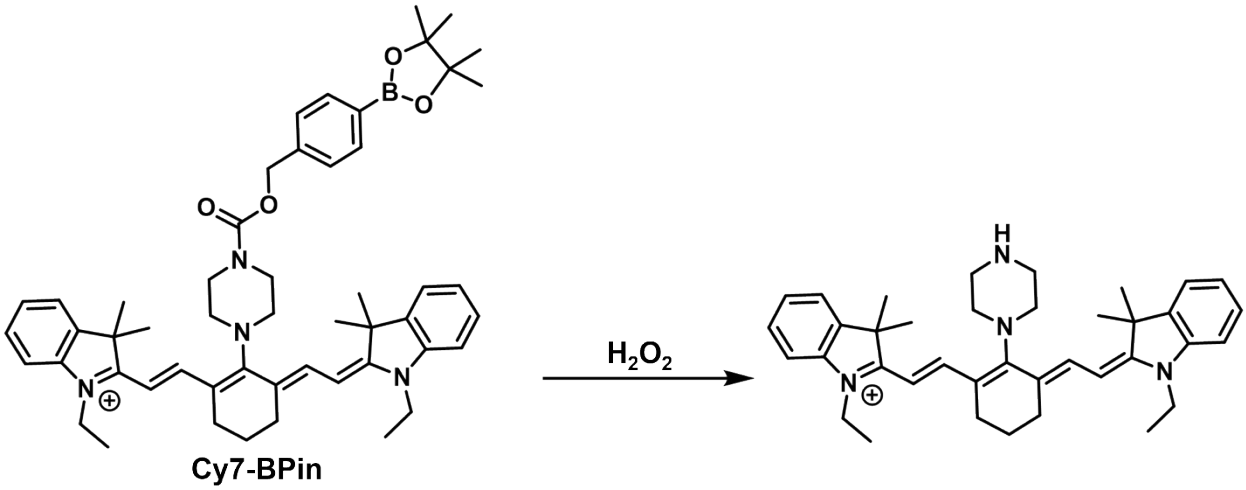


Figure S2. Absorption spectra of **Cy7-BPin** (20 μM) in PBS (pH = 7.20) before (**Blue**) and after (**Red**) addition of H_2O_2 (10 mM). The second measurement was recorded after 30 minutes incubation.



Scheme S3. Proposed H_2O_2 sensing mechanism of **Cy7-BPin**.

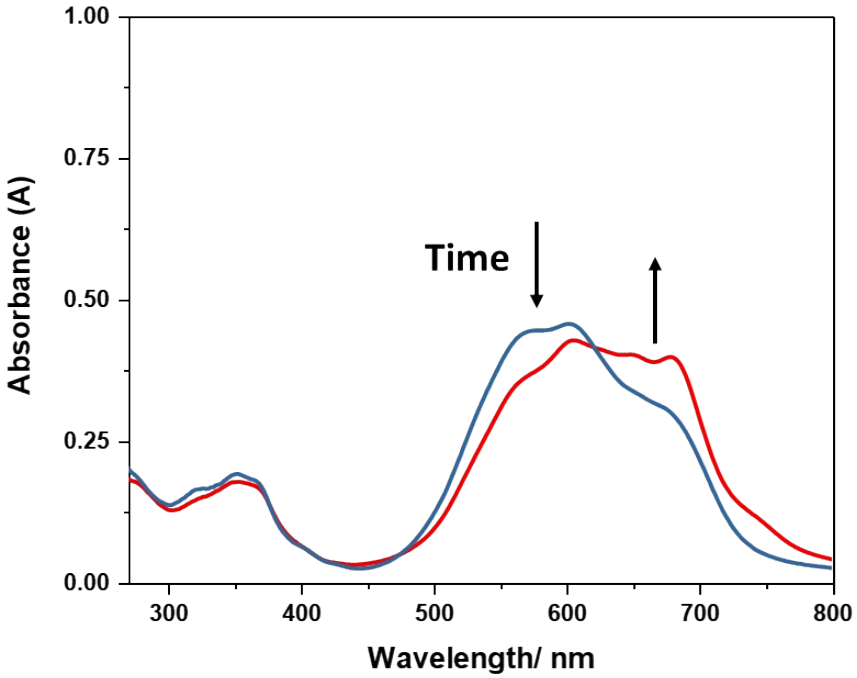
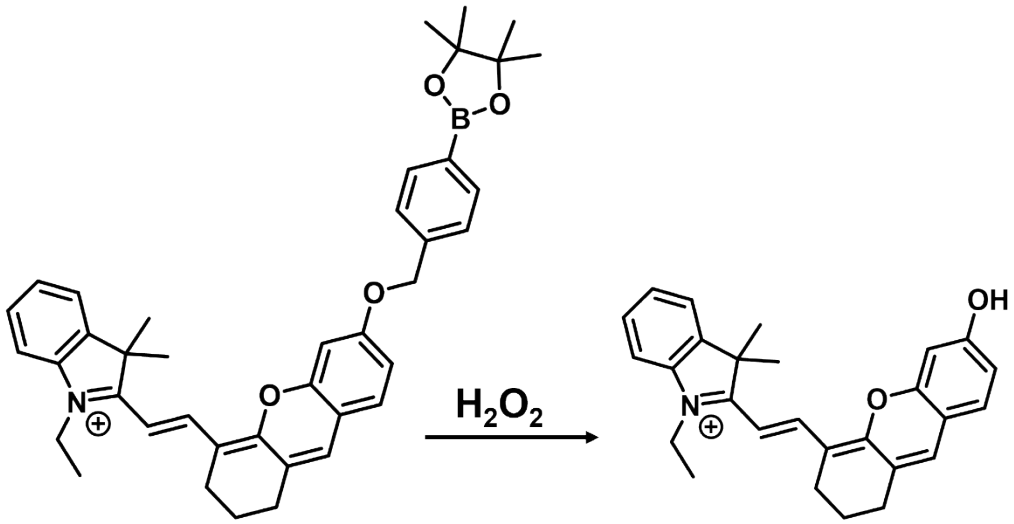


Figure S3. Absorption spectra of **HD-BPin** (20 μM) in PBS (pH = 7.20) before and after addition of H_2O_2 (200 μM). The second measurement was recorded after 30 minutes incubation.



Scheme S4. Proposed H_2O_2 sensing mechanism of **HD-BPin**.

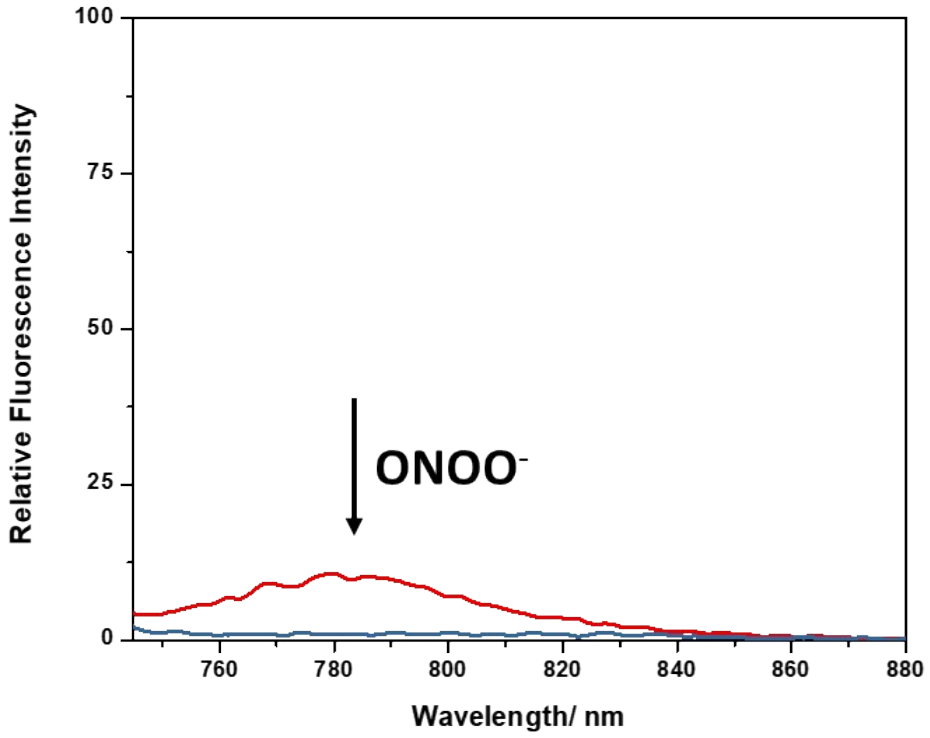


Figure S4. Fluorescence spectra of **Cy7-BPin** (10 μM) in PBS (pH = 7.20) before (**Red**) and after (**Blue**) the addition of $ONOO^-$ (10 μM) λ_{ex} = 730 nm (Slit widths: 10 nm and 10 nm).

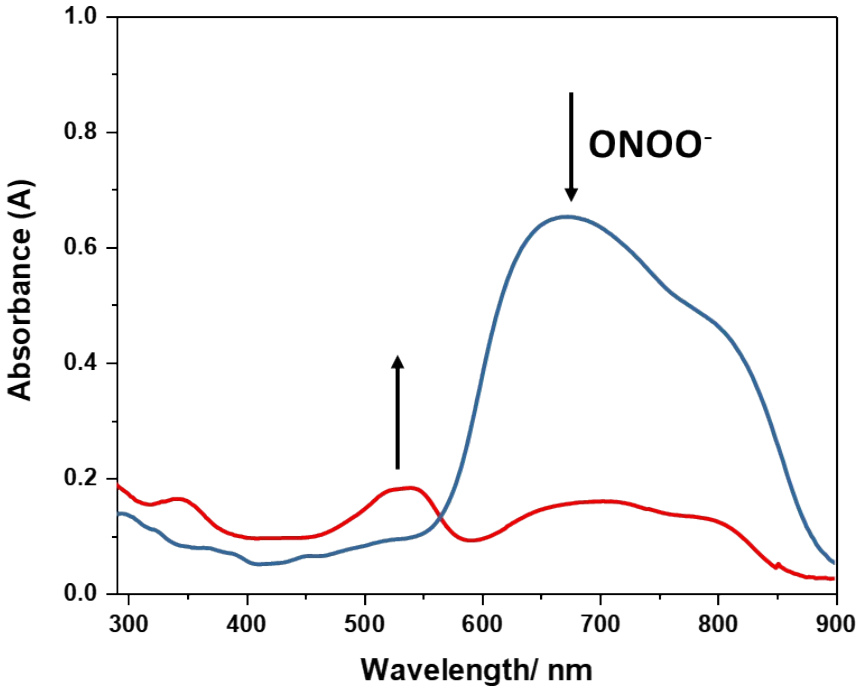


Figure S5. Absorption spectra of **Cy7-BPin** (20 μM) in PBS (pH = 7.20) before (**Blue**) and after (**Red**) addition of $ONOO^-$ (100 μM).

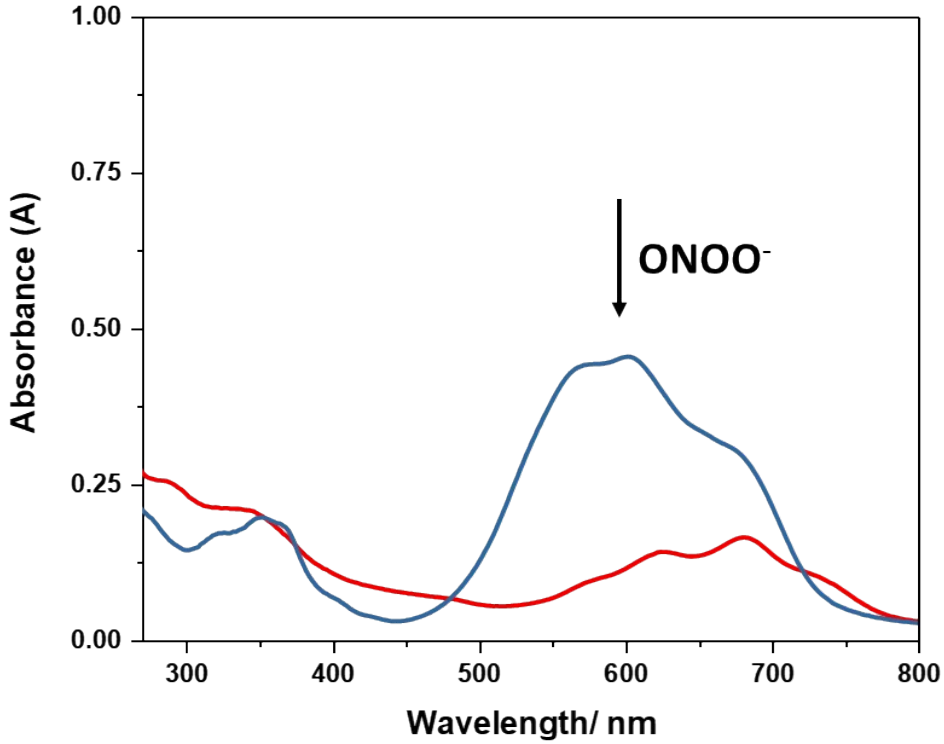


Figure S6. Absorption spectra of **HD-BPin** (20 μM) in PBS (pH = 7.20) before (**Blue**) and after (**Red**) addition of $ONOO^-$ (100 μM).

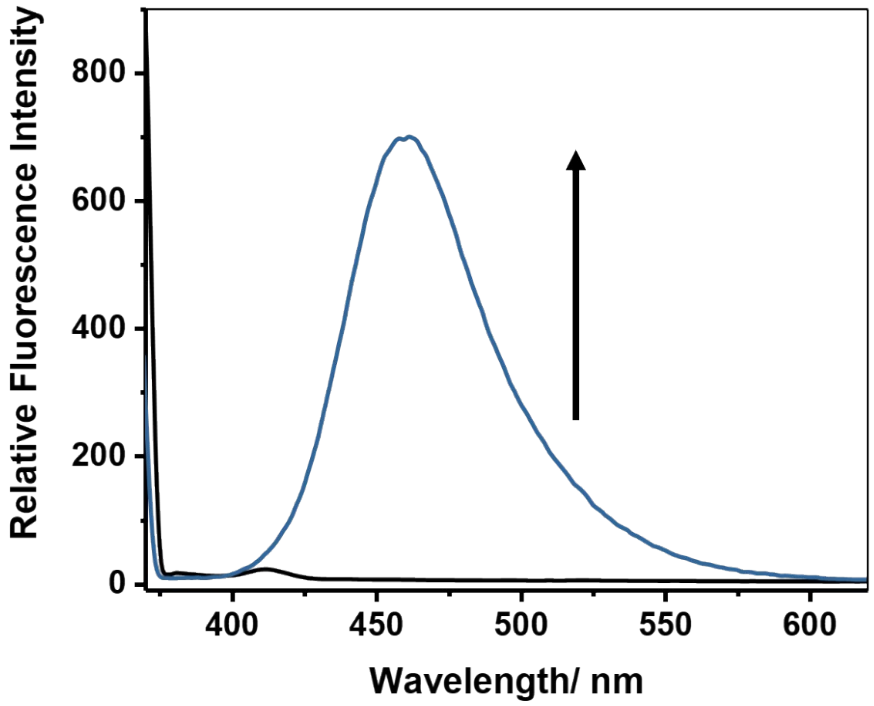
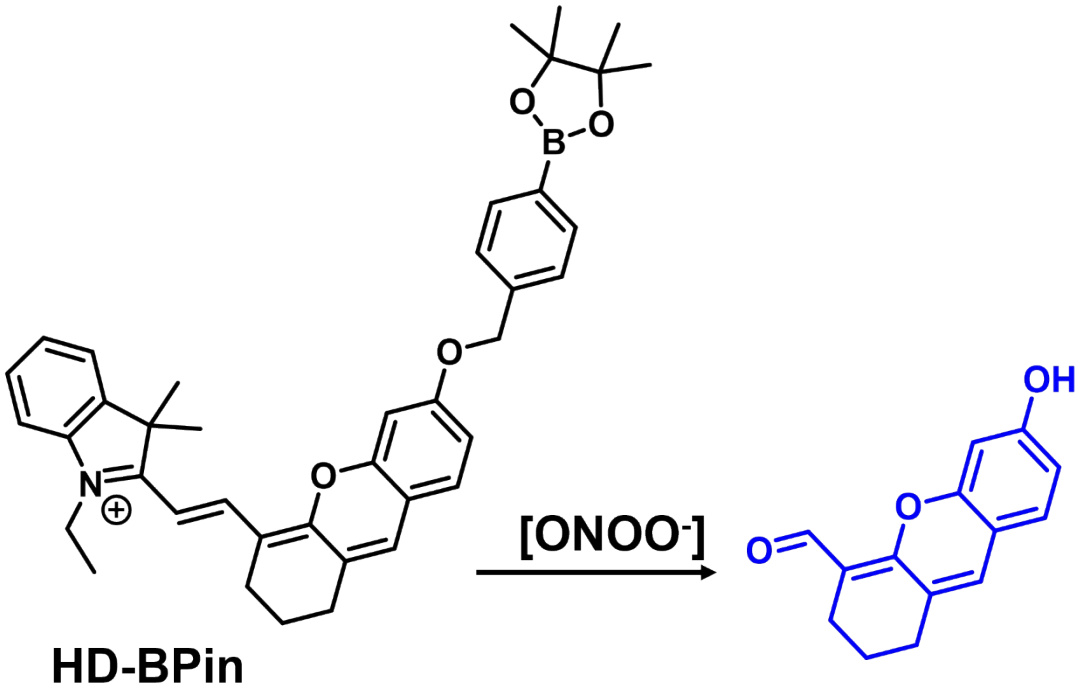


Figure S7. Fluorescence spectra of **HD-BPin** (10 μM) before and the addition of $ONOO^-$ (100 μM) in PBS (pH = 7.20). λ_{ex} = 360 nm (Slit widths: 10 nm and 5 nm).



Scheme S5. Proposed ONOO- sensing mechanism of **HD-BPin**.

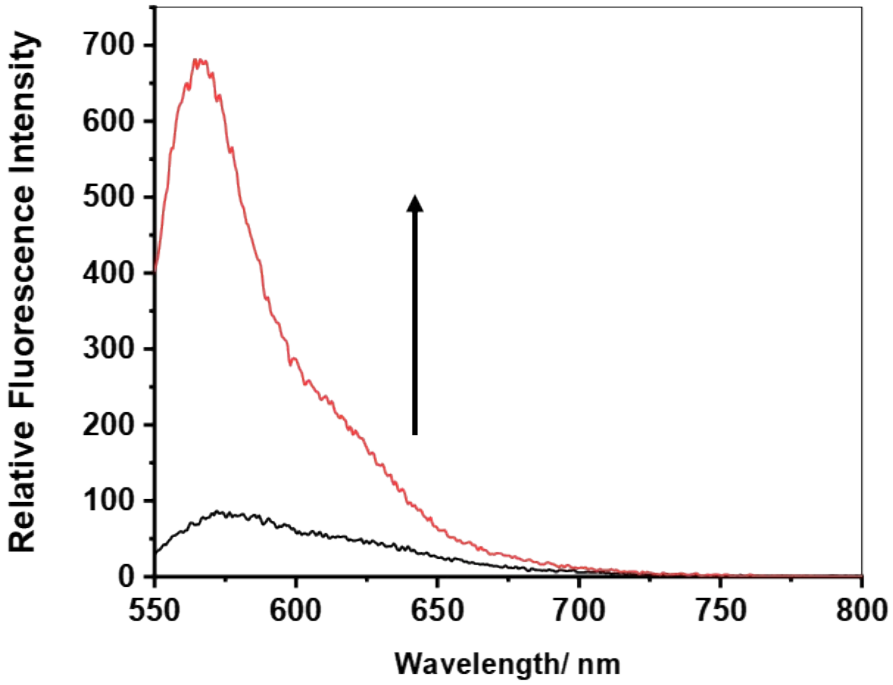


Figure S8. Fluorescence spectra of **PO1** (0.25 μM) before and the addition of H_2O_2 (1 mM) in PBS (pH = 7.20). λ_{ex} = 500 nm (Slit widths: 5 nm and 3 nm).

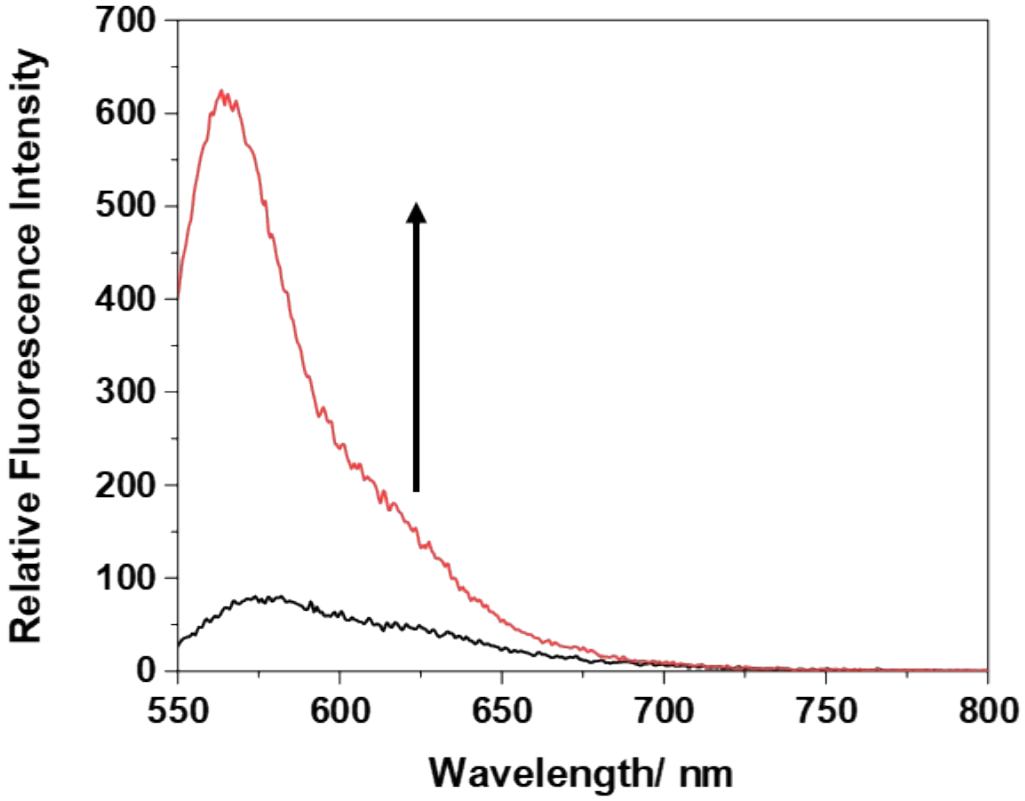


Figure S9. Fluorescence spectra of **PO1** (0.25 μM) before and the addition of $ONOO^-$ (2 μM) in PBS (pH = 7.20). λ_{ex} = 500 nm (Slit widths: 5 nm and 3 nm).

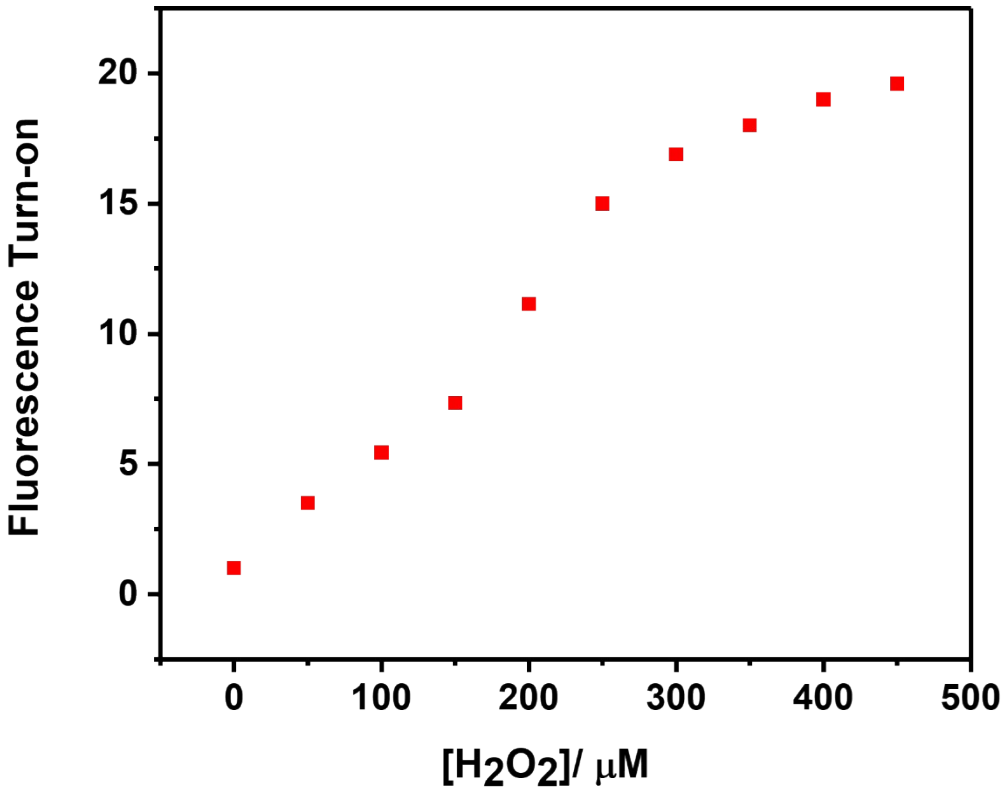


Figure S10. Fluorescence Turn-on of **HD-BPin** (10 μM) with increasing concentrations of H_2O_2 (0 - 450 μM) in PBS (pH = 7.20). λ_{ex} = 660 nm/ λ_{em} = 704 nm (Slit widths: 10 nm and 5 nm).

Limit of detection:

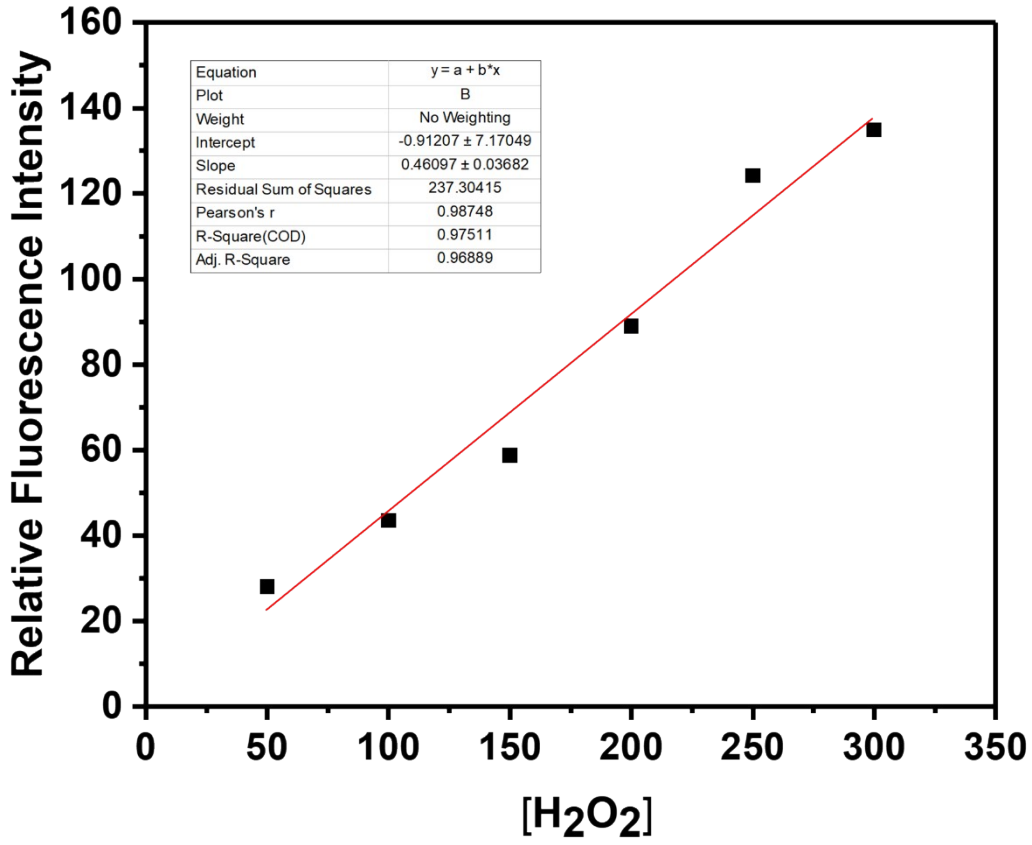


Figure S11. Relative fluorescence emission intensity of **HD-BPin** (10 μM) with increasing H_2O_2 concentrations (50 - 300 μM). Measurements were carried out in PBS (pH = 7.20). λ_{ex} = 660 nm/ λ_{em} = 704 nm (Slit widths: 10 nm and 5 nm).

Calculation for LOD = limit of detection (3σ/k). σ = standard deviation of the baseline measurement at 704 nm. k = slope.[4] LOD = 3 x 0.32/0.46 = 2.10 μM

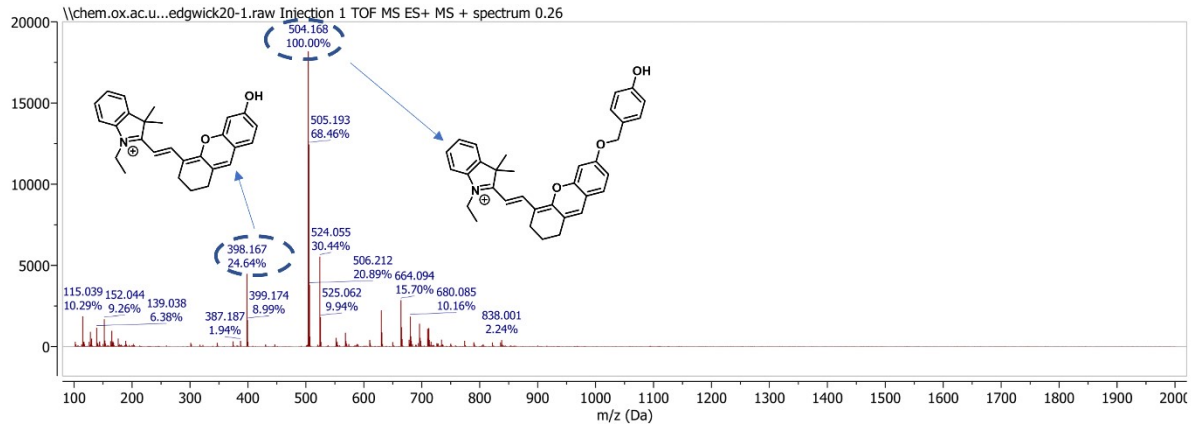


Figure S12. Mass spectrum of an aqueous solution of **HD-BPin** treated with excess H_2O_2 (ESI+).

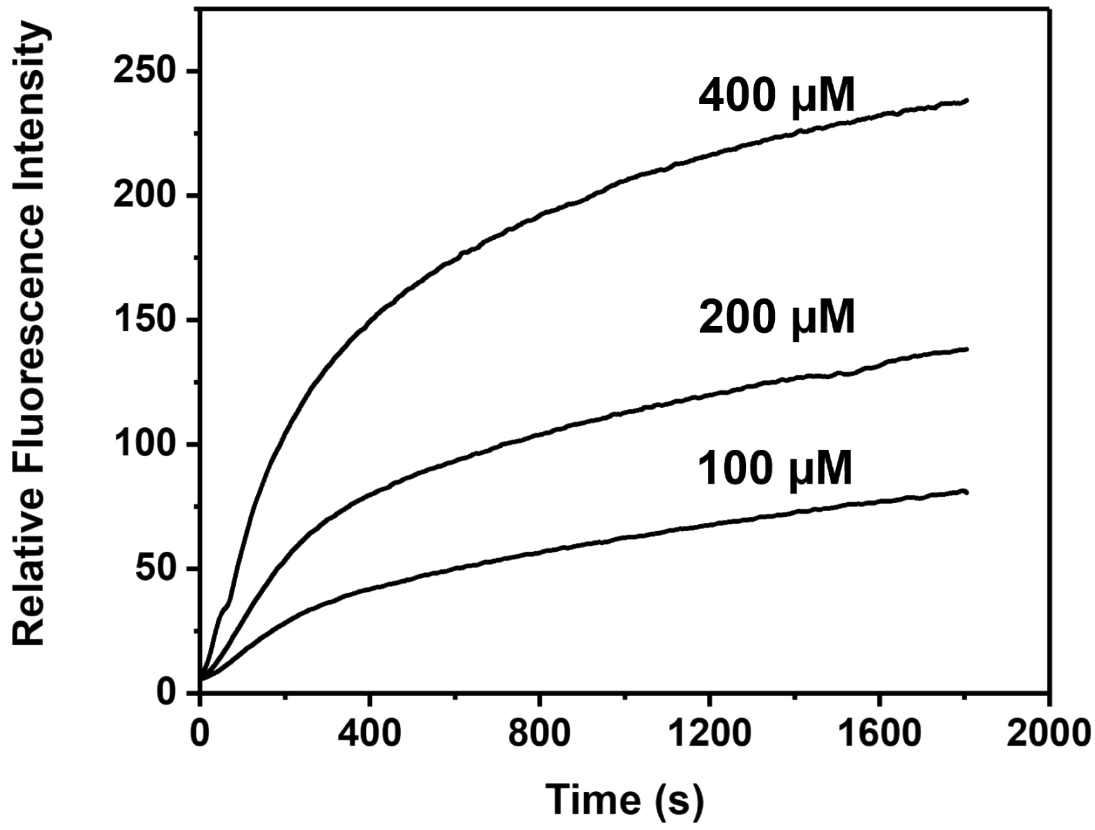


Figure S13. Changes in relative fluorescence emission intensity of **HD-BPin** (10 μM) at 704 nm with different concentrations of H_2O_2 (100 μM, 200 μM and 400 μM). All measurements were performed in PBS (pH = 7.20), λ_{ex} = 660 nm (Slit widths: 10 nm and 5 nm).

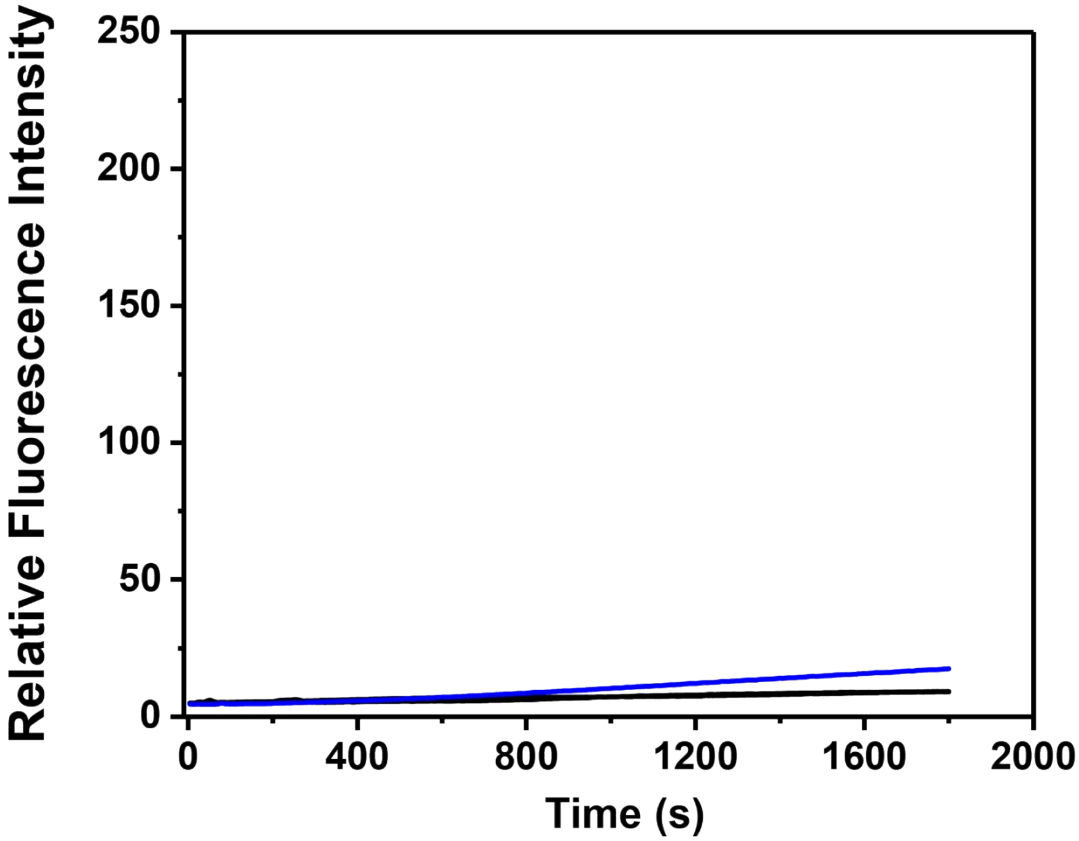


Figure S14. Changes in relative fluorescence emission intensity of **HD-BPin** (10 μM) at 460 nm with different concentrations of H_2O_2 (100 μM, 200 μM and 400 μM). All measurements were performed in PBS (pH = 7.20), λ_{ex} = 360 nm (Slit widths: 10 nm and 5 nm). Blue line with greatest intensity corresponds to 400 μM.

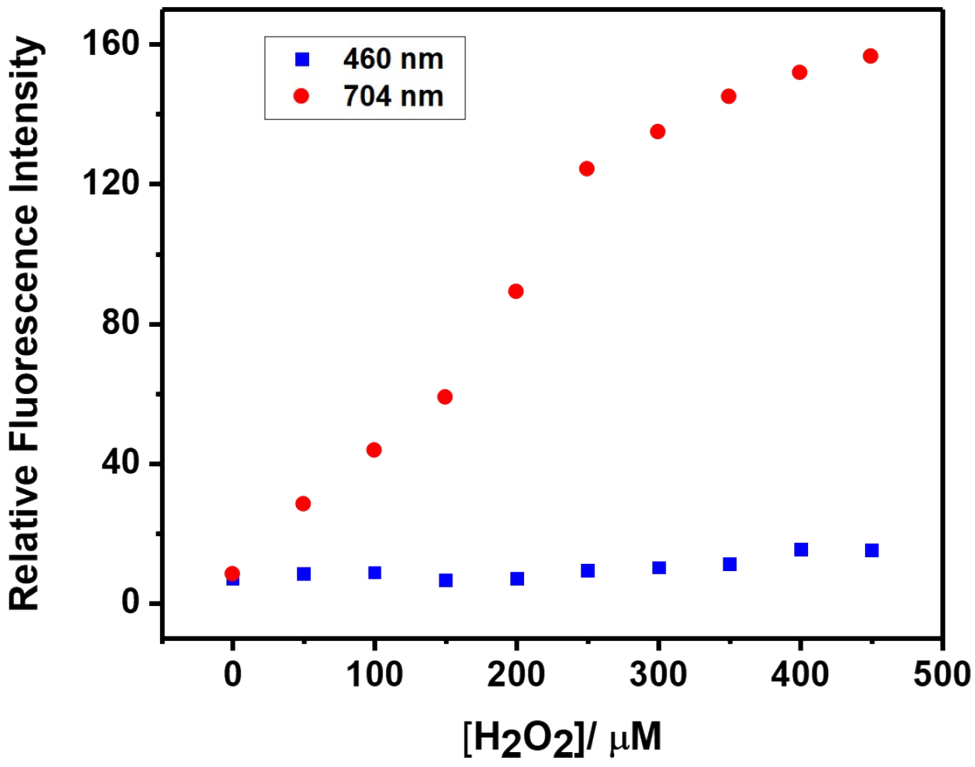


Figure S15. Relative fluorescence emission intensities of **HD-BPin** (10 μM) at 704 nm (**Red**) and 460 nm (**Blue**) with different concentrations of H_2O_2 (100 μM, 200 μM and 400 μM). All measurements were carried out in PBS (pH = 7.20). Emission at 704 nm - λ_{ex} = 660 nm (Slit widths: 10 nm and 5 nm). Emission at 460 nm - λ_{ex} = 360 nm (Slit widths: 10 nm and 5 nm).

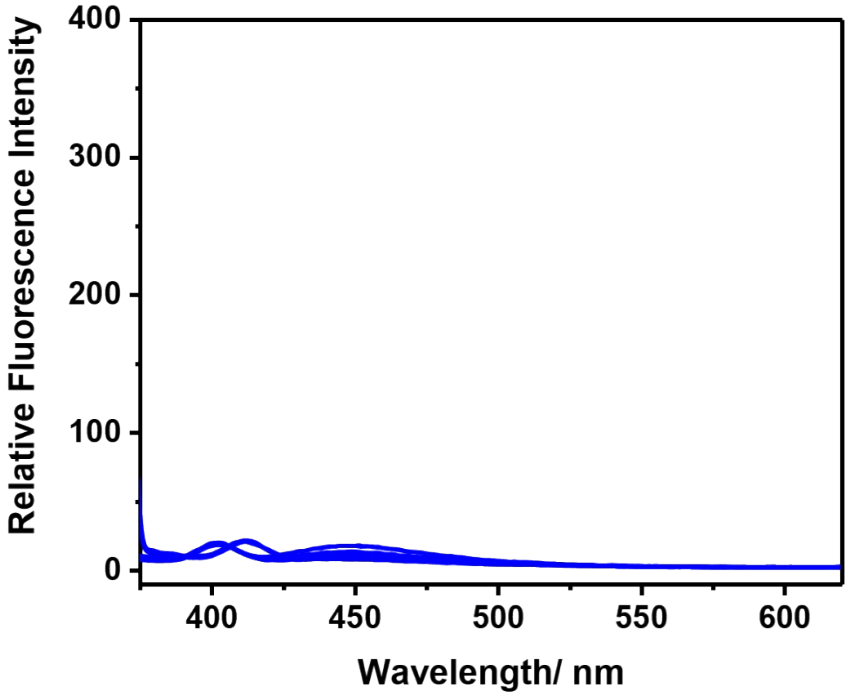


Figure S16. Fluorescence spectra of **HD-BPin** (10 μM) with different concentrations of H_2O_2 (100 μM, 200 μM and 400 μM). All measurements were carried out in PBS (pH = 7.20), λ_{ex} = 360 nm (Slit widths: 10 nm and 5 nm).

Limit of Detection:

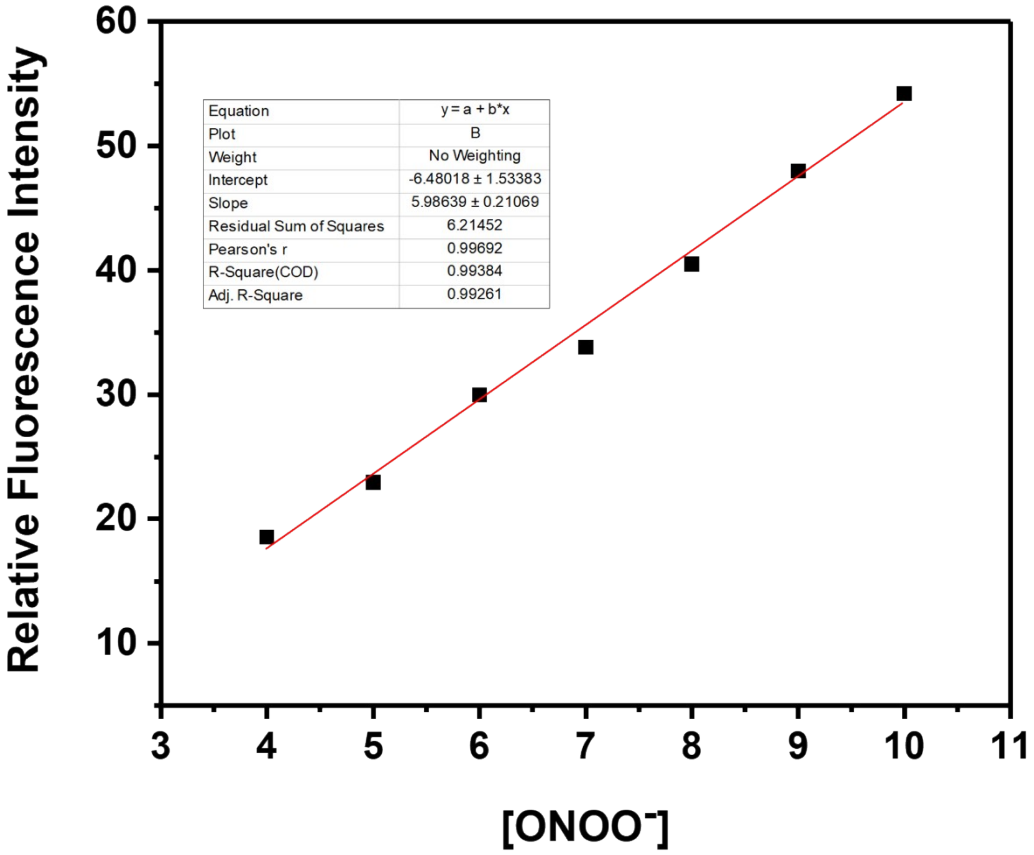


Figure S17. Relative fluorescence emission intensity of **HD-BPin** (10 µM) with $ONOO^-$ concentrations (4 - 10 µM). Measurements were carried out in PBS (pH = 7.20). λ_{ex} = 360 nm/ λ_{em} = 460 nm (Slit widths: 10 nm and 5 nm).

Calculation for LOD = limit of detection (3σ/k). σ = standard deviation of the baseline measurements at 460 nm. k = slope.[4] LOD = 3 x 0.55/5.99 = 0.28 µM

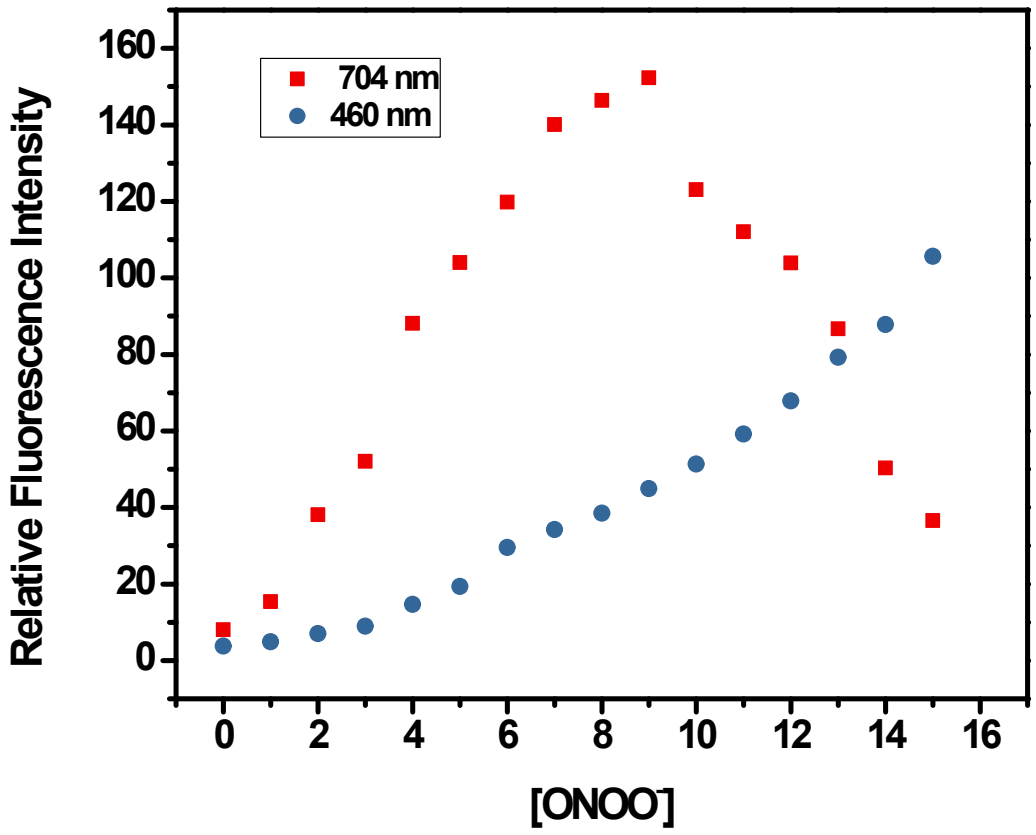


Figure S18. Relative fluorescence emission intensities of **HD-BPin** (10 μM) at 704 nm (**Red**) and 460 nm (**Blue**) with different concentrations of $ONOO^-$ (0 – 15 μM). All measurements were carried out in PBS (pH = 7.20). Emission at 704 nm - λ_{ex} = 660 nm (Slit widths: 10 nm and 5 nm). Emission at 460 nm - λ_{ex} = 360 nm (Slit widths: 10 nm and 5 nm).

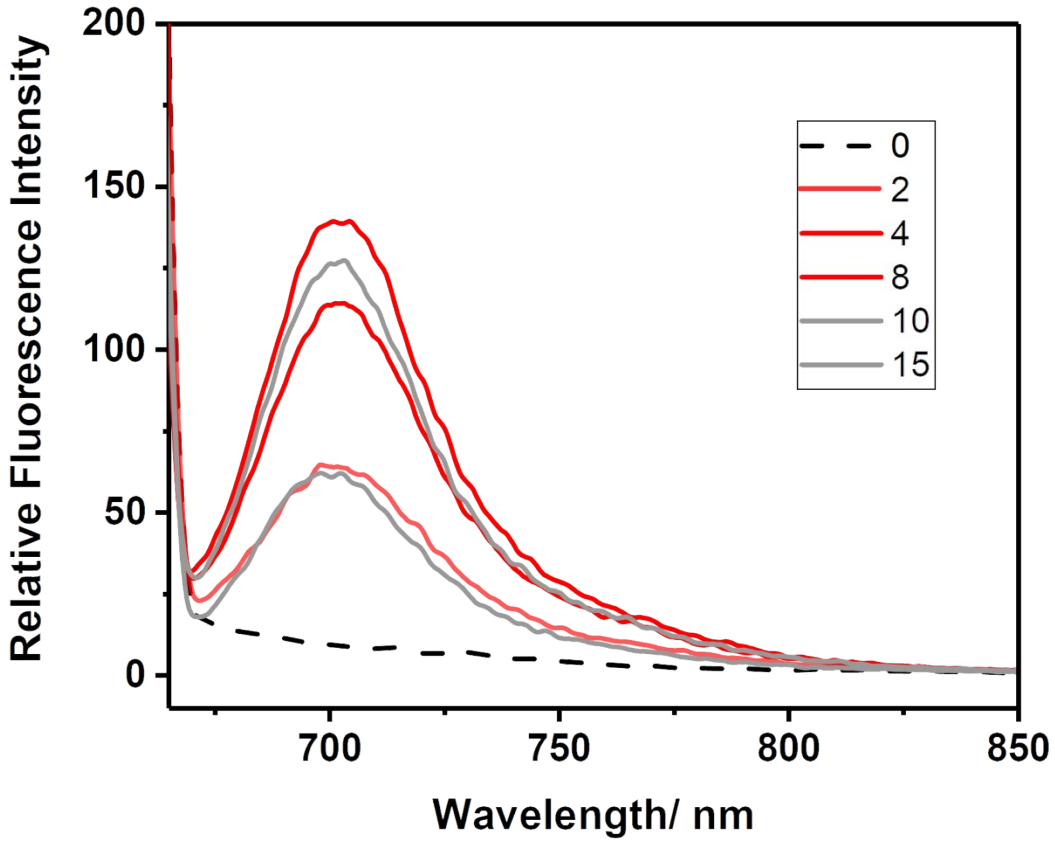


Figure S19. Fluorescence spectra of **HD-BPin** (10 µM) with increasing concentrations of $ONOO^-$ (0, 2, 4, 8, 10 and 15 µM, λ_{ex} = 660 nm). All measurements were carried out in PBS (pH = 7.20). Red line indicates increase in fluorescence intensity. Grey Line indicates the subsequent decrease in fluorescence intensity.

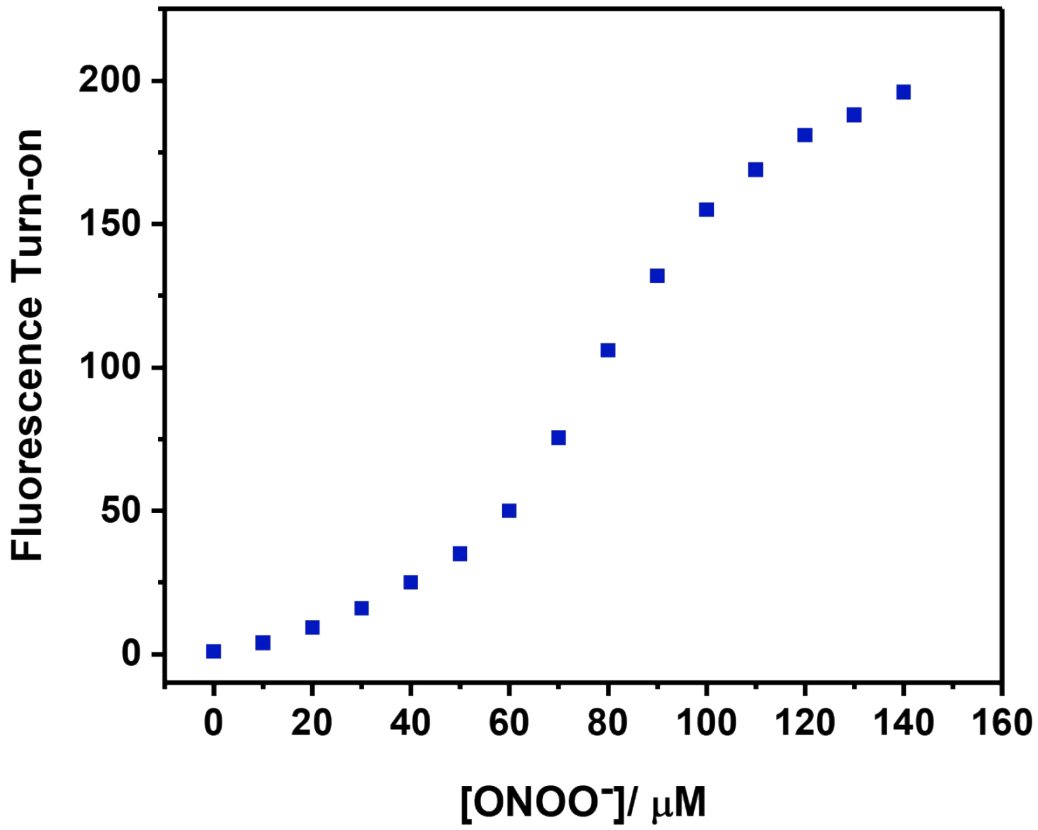
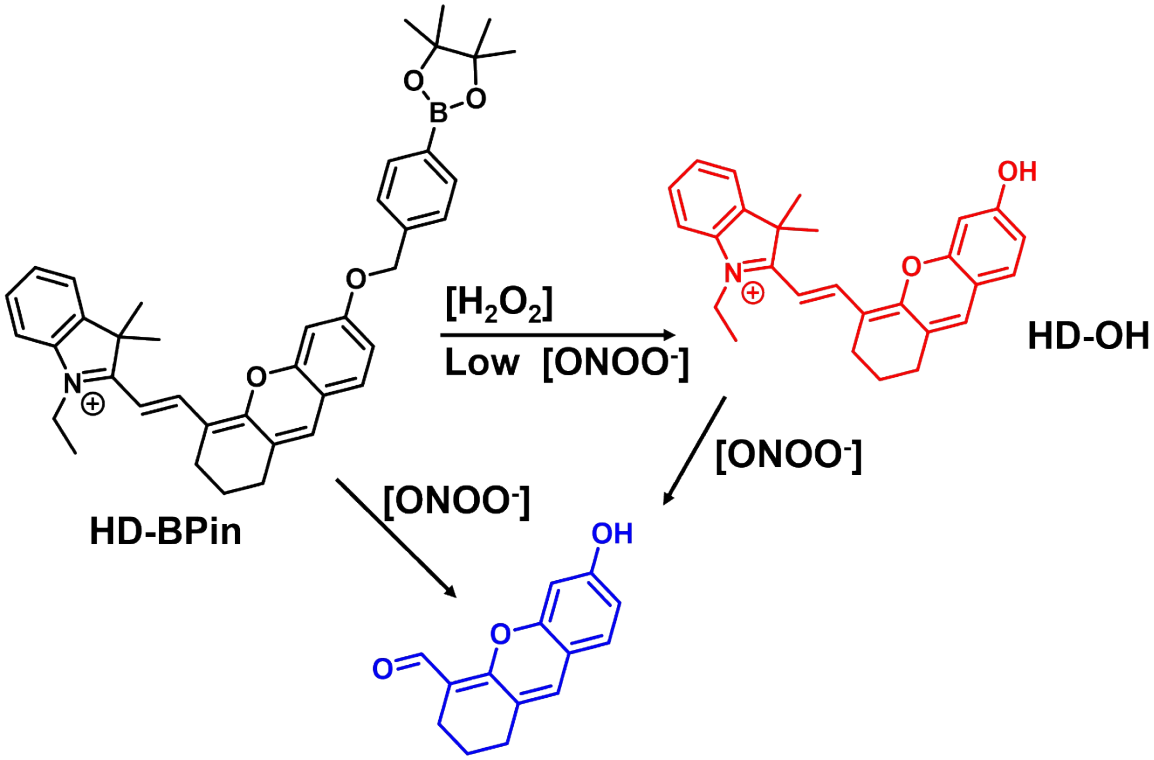


Figure S20. Fluorescence Turn-on of **HD-BPin** (10 μM) with increasing concentrations of $ONOO^-$ (0 – 140 μM) in PBS (pH = 7.20). λ_{ex} = 360 nm/ λ_{em} = 460 nm (Slit widths: 10 nm and 5 nm).



Scheme S6. Proposed ONOO- sensing mechanism of **HD-BPin.**

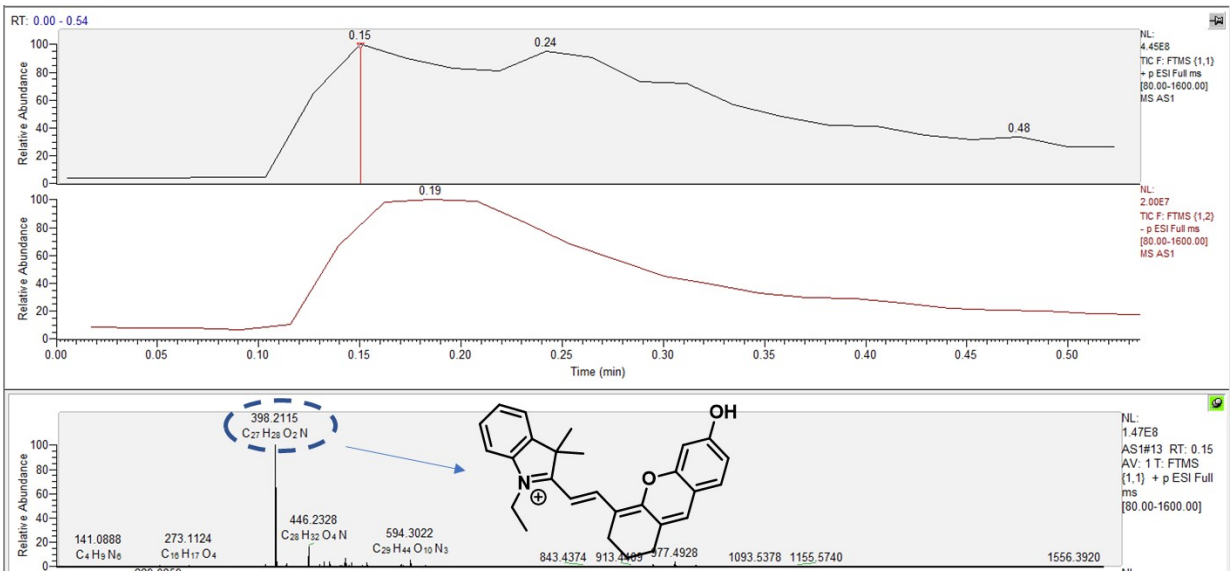


Figure S21. Mass spectrum of an aqueous solution of **HD-BPin** treated with $ONOO^-$ (ESI+).

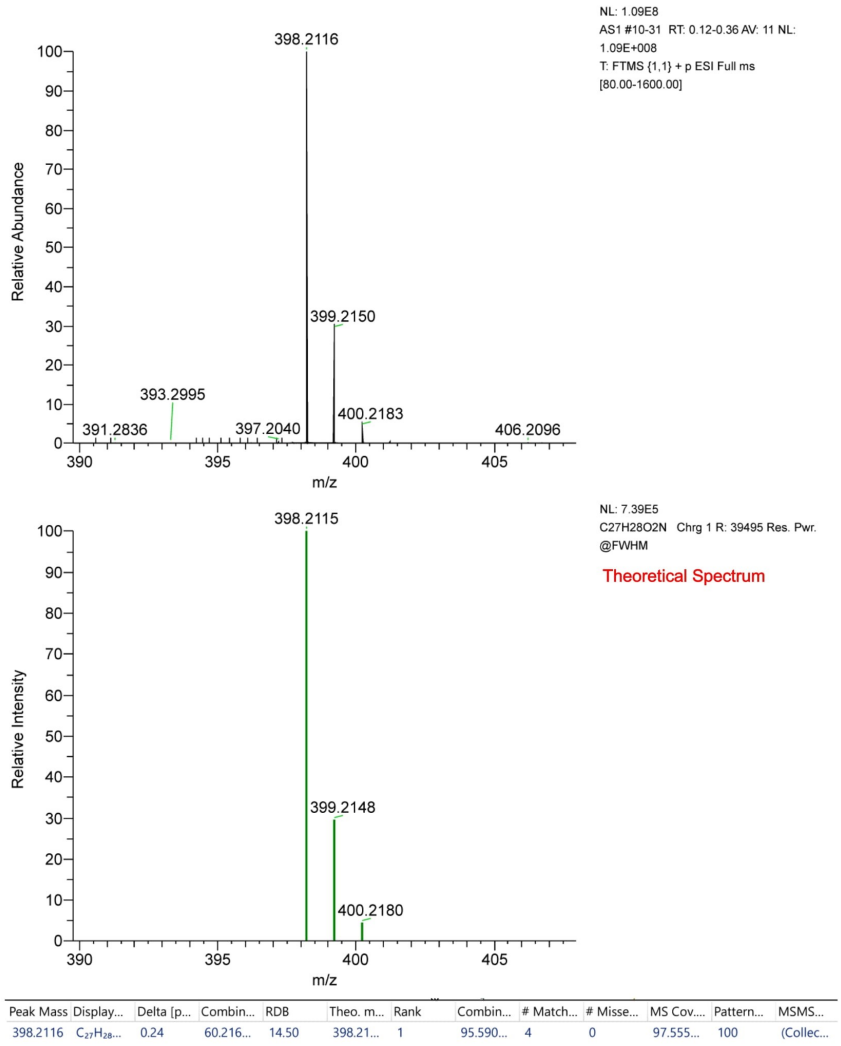


Figure S22. High-resolution mass spectrum of an aqueous solution of **HD-BPin** treated with $ONOO^-$ to confirm presence of red emissive species (ESI+).

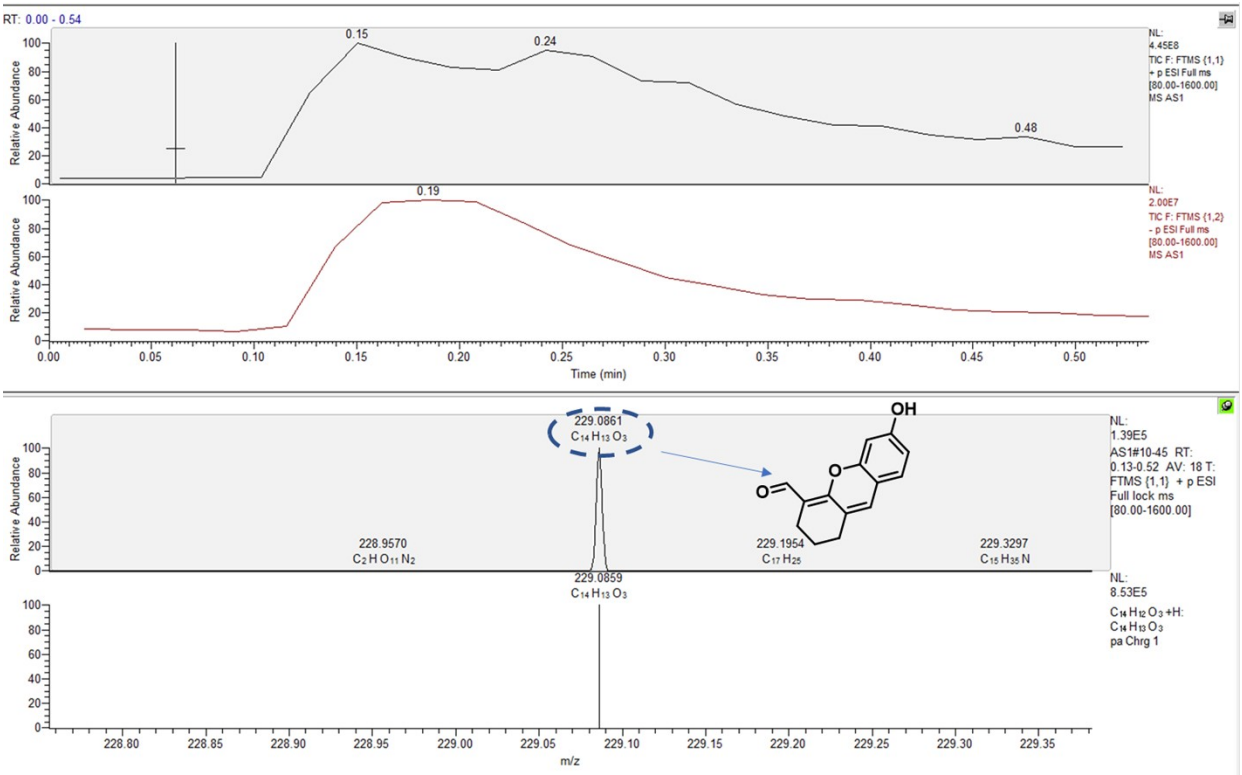
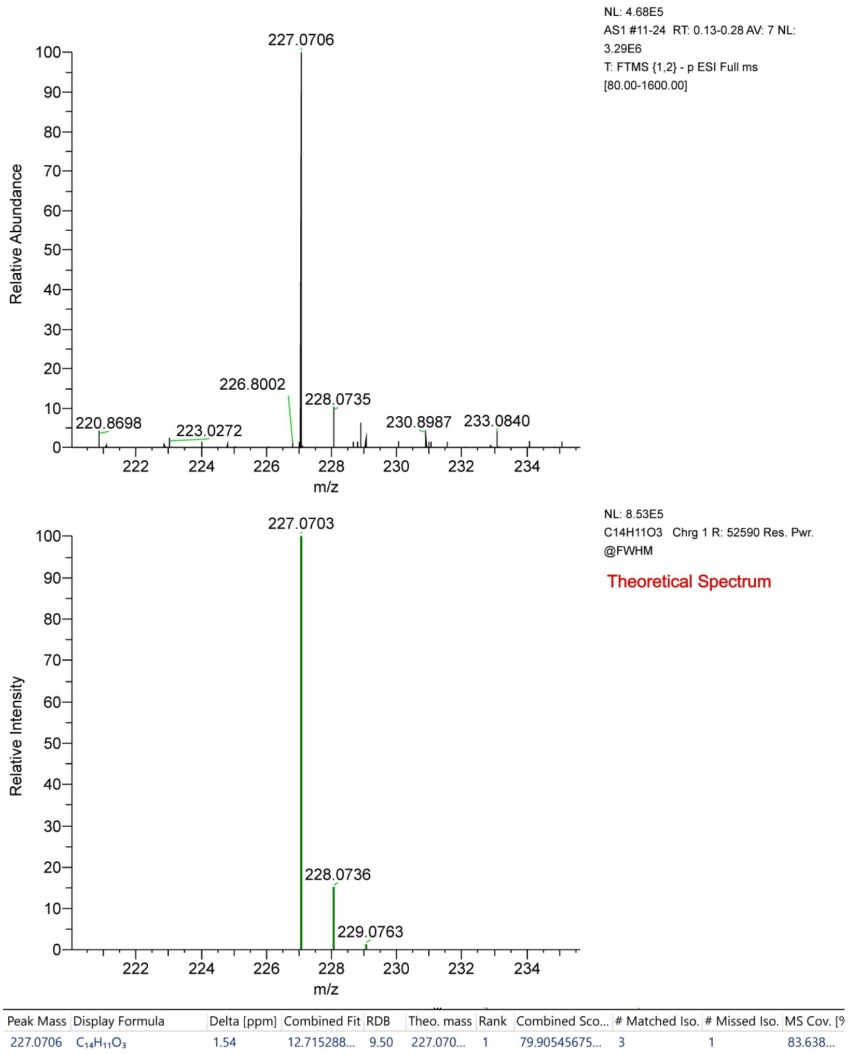


Figure S23. Mass spectrum of an aqueous solution of **HD-BPin** treated with $ONOO^-$ (ESI+). Note – the relative intensity of the blue product is lower compared to that of the red product.



Peak Mass	Display Formula	Delta [ppm]	Combined Fit	RDB	Theo. mass	Rank	Combined Sco...	# Matched Iso.	# Missed Iso.	MS Cov. [9
227.0706	$C_{14}H_{11}O_3$	1.54	12.715288...	9.50	227.070...	1	79.90545675...	3	1	83.638...

Figure S24. High-resolution mass spectrum of an aqueous solution of **HD-BPin** treated with $ONOO^-$ to confirm the presence of the blue emissive species (ESI-).

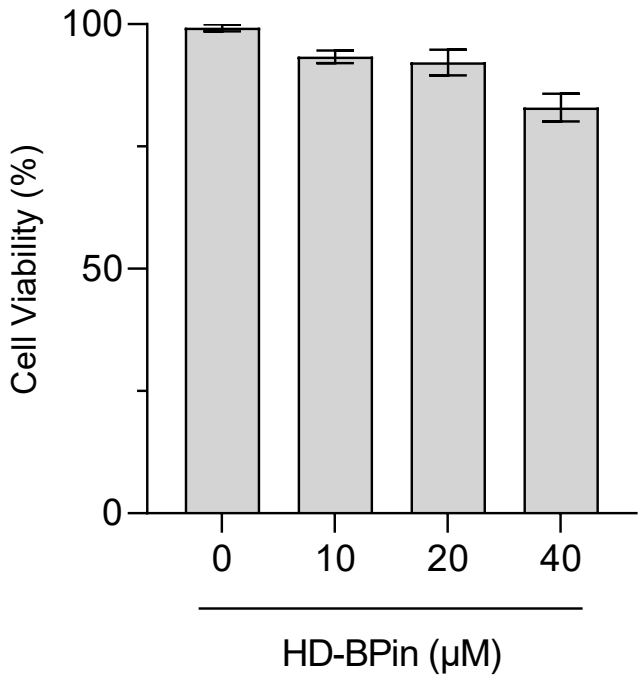


Figure S25. Cell toxicity of **HD-BPin** in A549 cells. A549 cells were treated with the indicated concentrations of **HD-BPin** for 30 minutes. **HD-BPin** was removed and an MTT assay was carried out 24 hours post treatment to assess cell viability. Data presented as % cell viability relative to untreated control. Error bars represent SD.

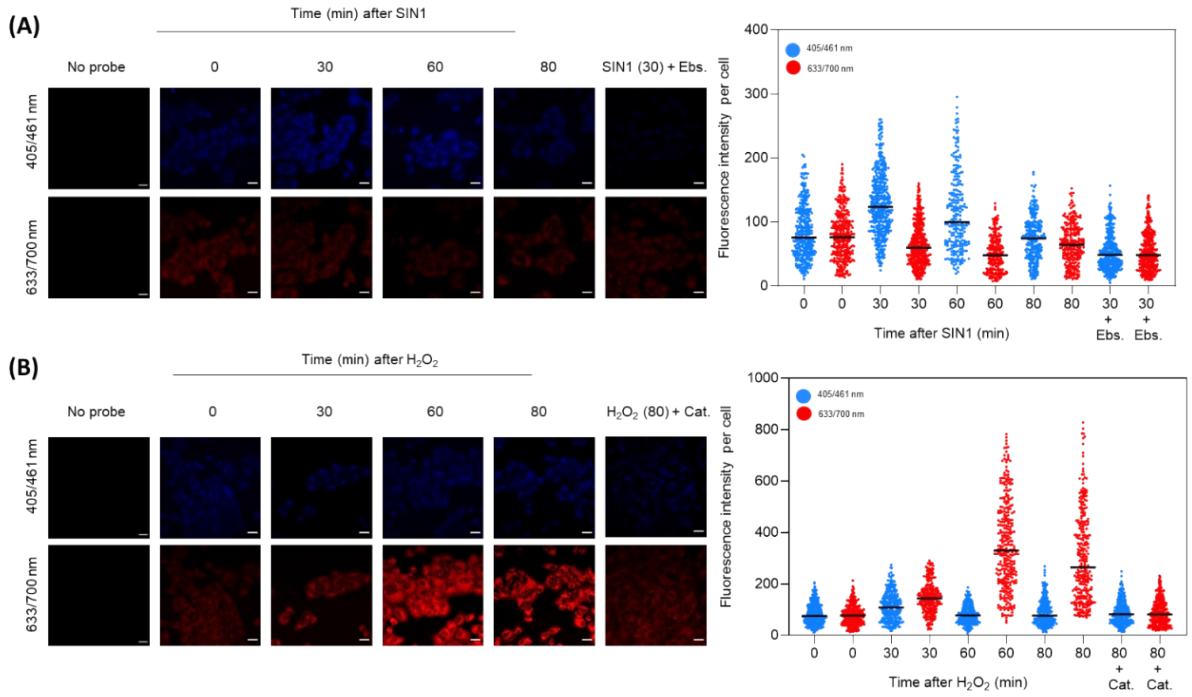


Figure S26. Response of **HD-BPin** to exogenous $ONOO^-$ and H_2O_2. H460 cells were pre-treated with HD-BPin (10 µM), treated with either SIN1 (500 µM) or H_2O_2 (100 µM) and fixed at the indicated times. Cells were also pre-treated for 4.5 hours with either Ebselen (10 µM) to scavenge $ONOO^-$ or Catalase (1000 U/mL) to scavenge H_2O_2. A. representative images of time points taken at 405/461 nm to visualize changes in blue emission and 633/700 nm to visualize changes in red emission. Scale bar represents 20 µm. Images taken at 63x magnification B. Quantification of experiment in A. Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1

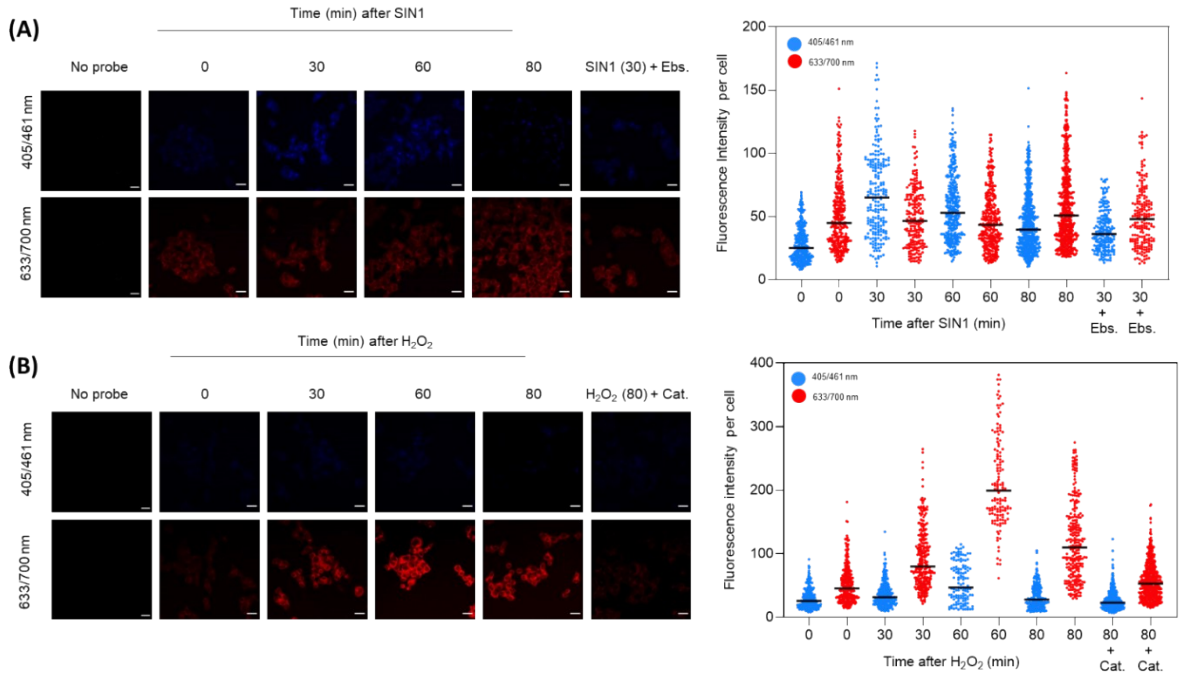


Figure S27. Response of **HD-BPin** to exogenous $ONOO^-$ and H_2O_2. HCT116 cells were pre-treated with **HD-BPin** (10 µM), treated with either SIN1 (500 µM) or H_2O_2 (100 µM) and fixed at the indicated times. Cells were also pre-treated for 4.5 hours with either Ebselen (10 µM) to scavenge $ONOO^-$ or Catalase (1000 U/mL) to scavenge H_2O_2. A. representative images of time points taken at 405/461 nm to visualize changes in blue emission and 633/700 nm to visualize changes in red emission. Scale bar represents 20 µm. Images taken at 63x magnification B. Quantification of experiment in A. Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1

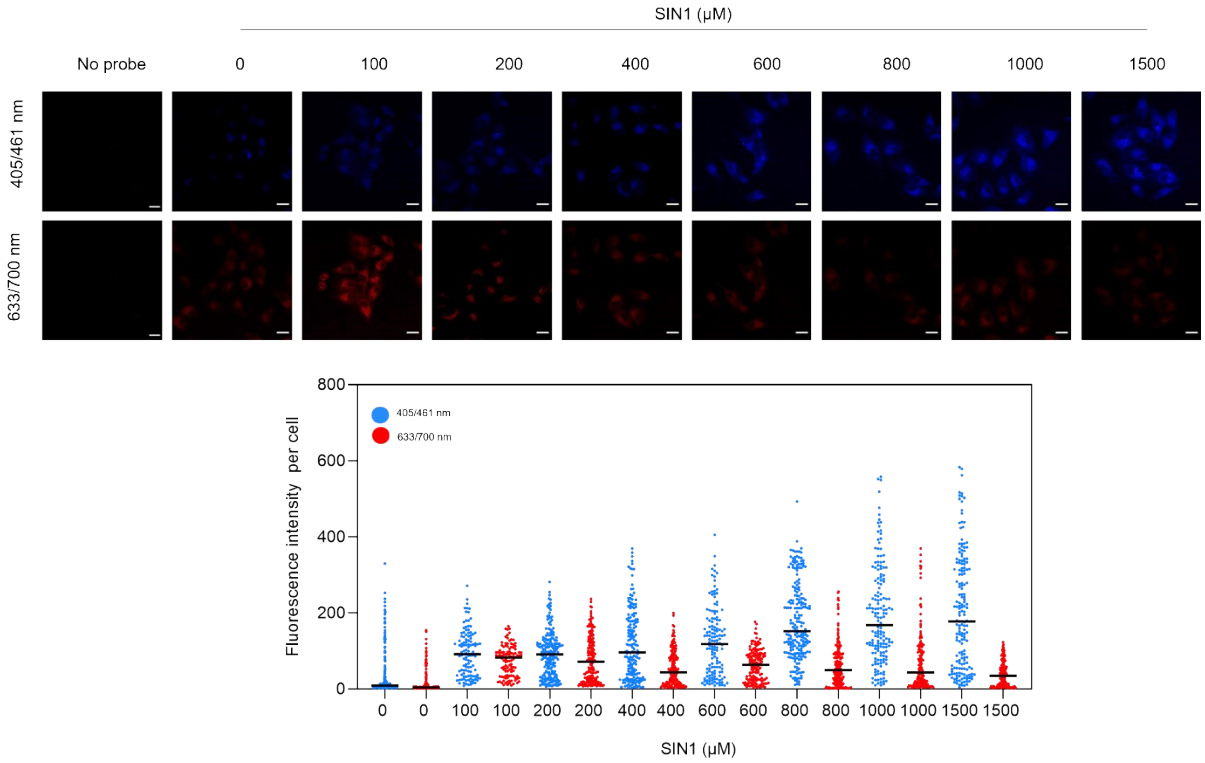


Figure S28. Fluorescence images of A549 cells incubated with **HD-BPin** (10 μM) followed by increasing concentrations of SIN-1 (0 – 1500 μM). A549 cells were pre-treated with **HD-BPin** (10 μM) and then treated with SIN-1 and fixed after 30 mins. Top: Representative images of time points taken at 405/461 nm and 633/700 nm. Scale bar represents 20 μm. Images taken at 63x magnification. Bottom: Quantification of experiment in A. Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1.

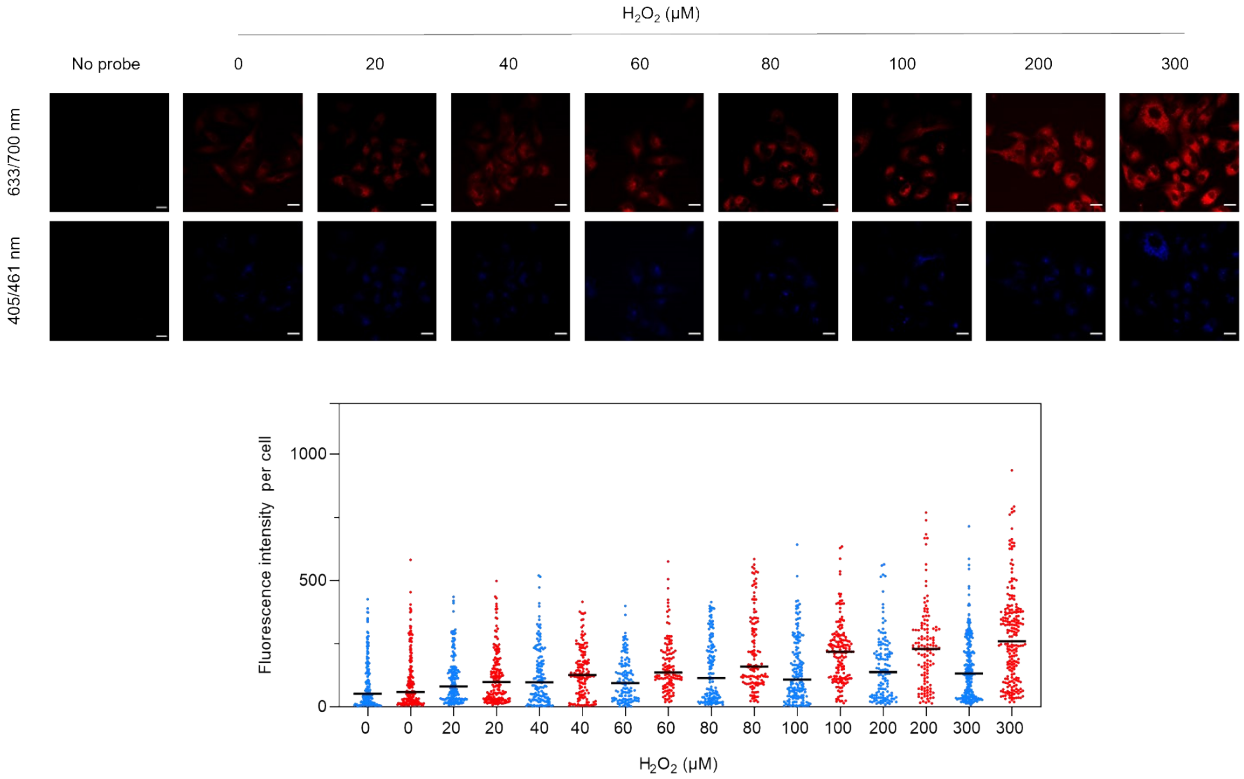


Figure S29. Fluorescence images of A549 cells incubated with **HD-BPin** (10 μM) followed by increasing concentrations of H_2O_2 (0 – 300 μM). A549 cells were pre-treated with **HD-BPin** (10 μM) and then treated with H_2O_2 and fixed after 80 mins. Top: representative images of time points taken at 405/461 nm and 633/700 nm. Scale bar represents 20 μm. Images taken at 63x magnification. Bottom: Quantification of experiment in A. Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1.

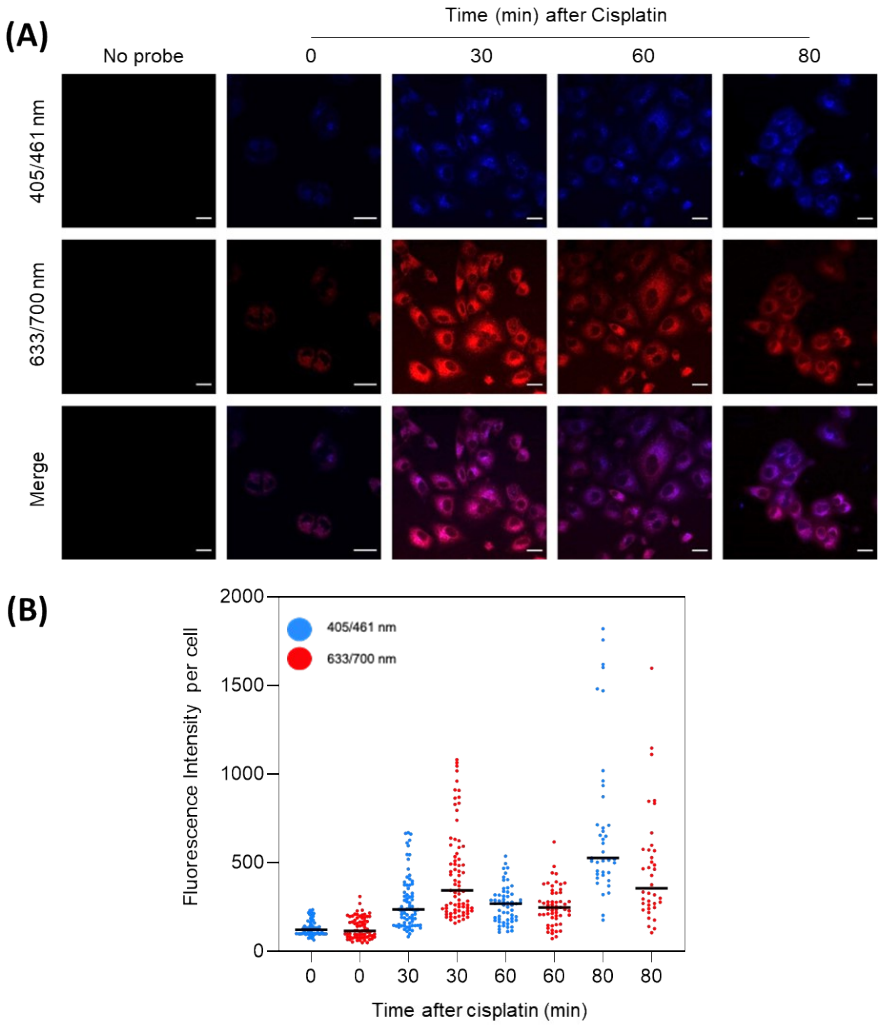


Figure S30. Fluorescence imaging of **HD-BPin** with the addition of cisplatin in A549 cells. A549 cells were pre-treated with **HD-BPin** (10 mM) for 30 minutes and then treated with cisplatin (15 µM). Cells were fixed at the indicated times (min). (A) Representative fluorescence images of time points taken at 405/461 nm and 633/700 nm. Scale bar represents 20 µm. (B) Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1

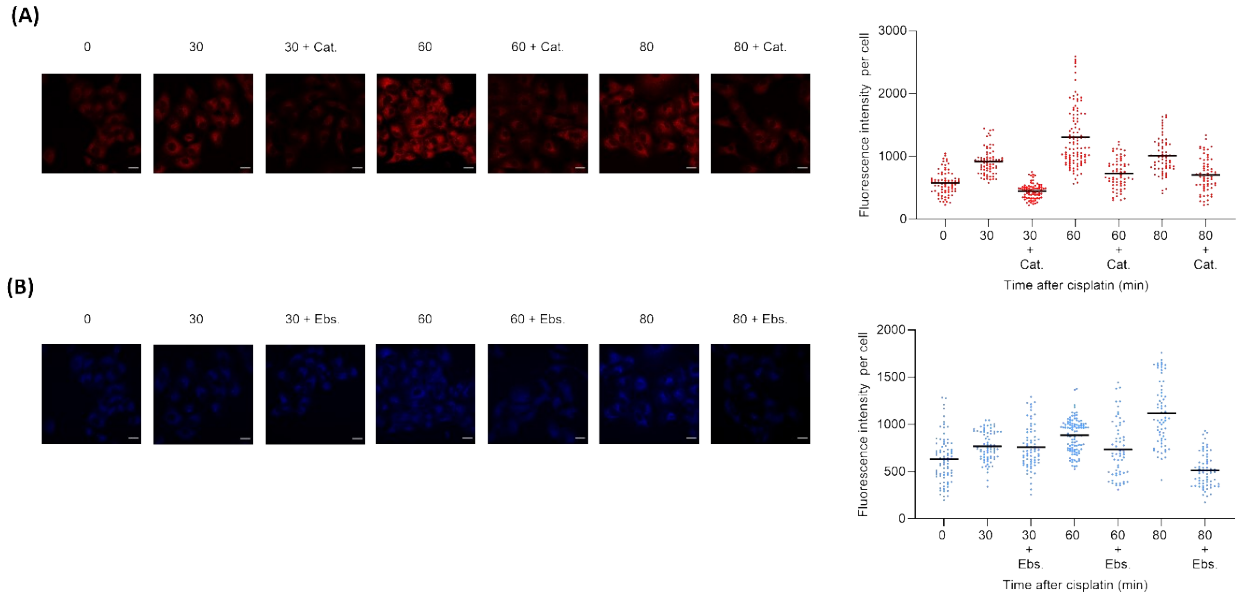


Figure S31. Fluorescence imaging of **HD-BPin** with the addition of cisplatin in A549 cells. A549 cells were pre-treated with **HD-BPin** (10 µM) for 30 minutes and then treated with cisplatin (15 µM) with and without 4-hour pre-treatment of Catalase (Cat.) (1000 U/mL) to scavenge H_2O_2 or Ebselen (Ebs.) (10 µM) to scavenge $ONOO^-$. Cells were fixed at the indicated times (min). (A) Representative fluorescence images and plotted data of fluorescence intensity per cell of time points taken at 633/700 nm to visualize changes in red emission (B) Representative fluorescence images and plotted data of fluorescence intensity per cell of time points taken at 405/461 nm to visualize changes in blue emission. Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1. Exogenous catalase addition has previously been shown to reduce intracellular H_2O_2.[5-8]

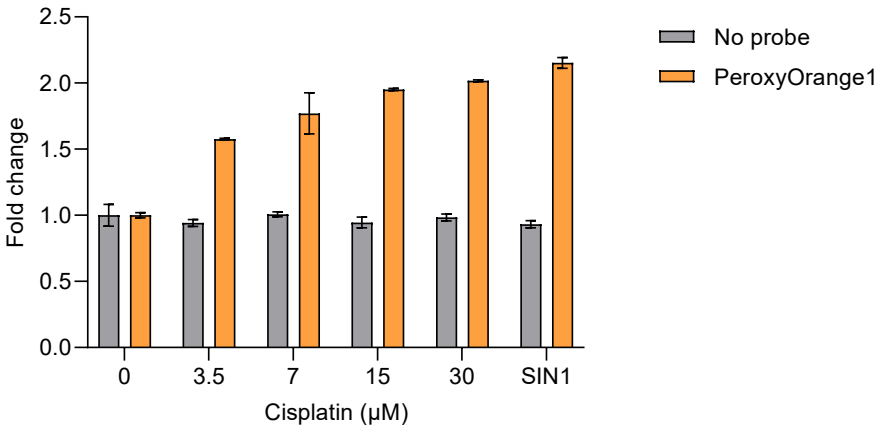


Figure S32. A549 cells were treated with indicated concentrations of cisplatin for 80 minutes. In the final 40 minutes of cisplatin incubation, PeroxyOrange1 (5 μM) was added to cells. SIN1 (500 μM) was used as a positive control. Fluorescent intensity was measured using a CLARIOstar plate reader (543/545-750 nm). Data plotted are fold change compared to untreated control. n=1

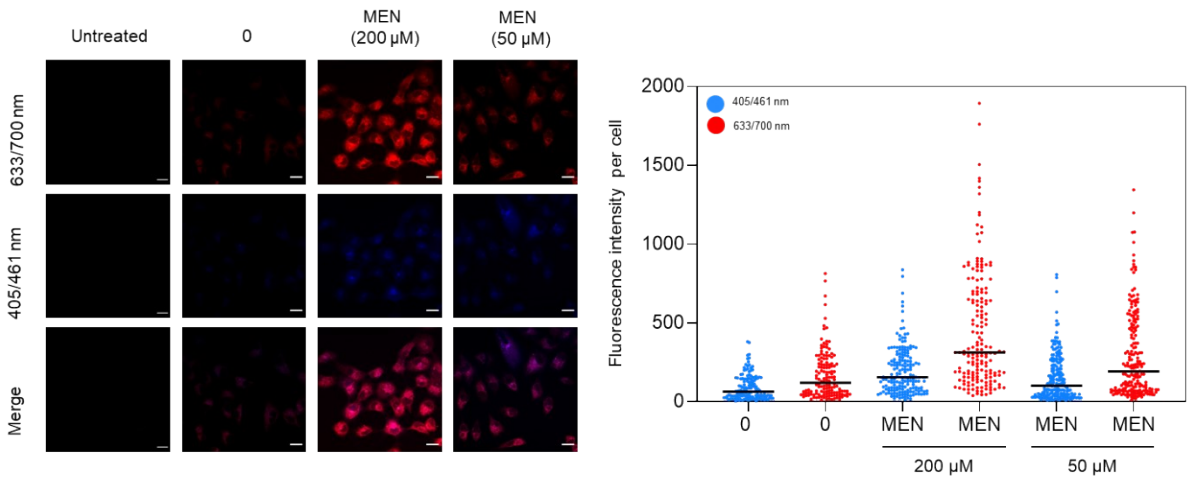


Figure S33. Response of **HD-BPin** to Menadione (MEN). A549 cells were pre-treated with **HD-BPin** (10 µM), treated with indicated concentrations of Menadione and fixed at 60 mins following addition of Menadione A. Left: Representative images **HD-BPin** time points taken at 405/461 nm to visualise blue emission and 633/700 nm to visualise red emission. Right: Quantification of panel A. Scale bar represents 20 µm. Images taken at 63x magnification Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1

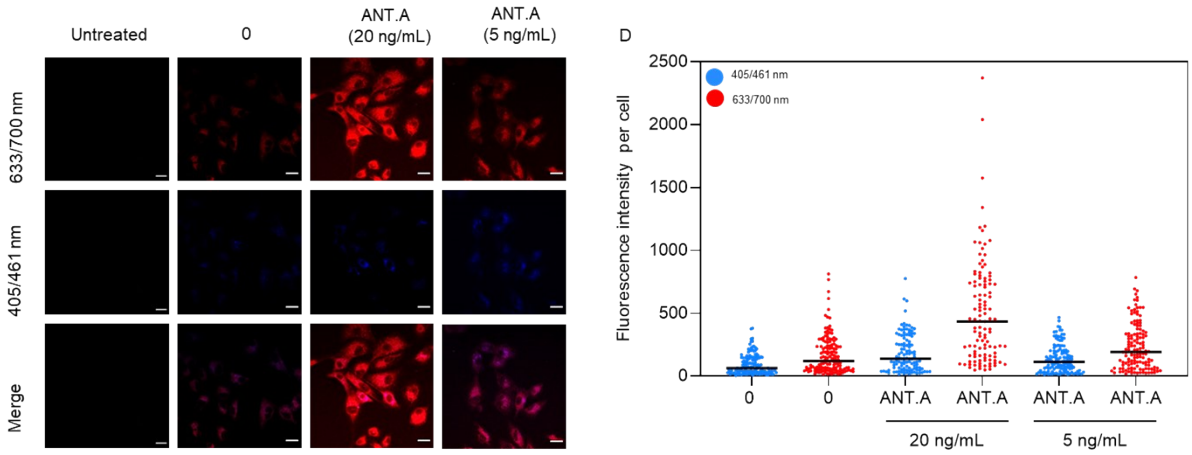
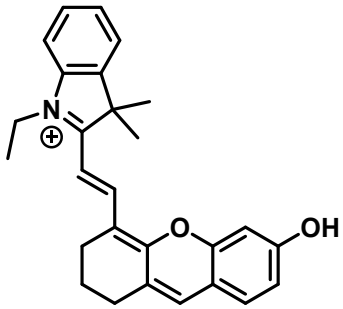


Figure S34. Response of **HD-BPin** to Antimycin A (ANT.A). A549 cells were pre-treated with **HD-BPin** (10 µM), treated with indicated concentrations of Antimycin A and fixed at 60 mins. Left: Representative images **HD-BPin** time points taken at 405/461 nm to visualize blue emission and 633/700 nm to visualize red emission. Right: Quantification of panel A. Scale bar represents 20 µm. Images taken at 63x magnification Data plotted are fluorescence intensity per cell, black line indicates mean. For each condition 100 cells were imaged and quantified. Outliers were excluded using Tukeys box plot. n=1

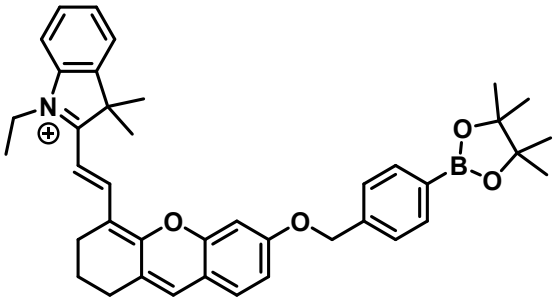
4. Chemical Synthesis of HD-BPin and Cy7-BPin

(*E*)-1-ethyl-2-(2-(6-hydroxy-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium (HD-OH)



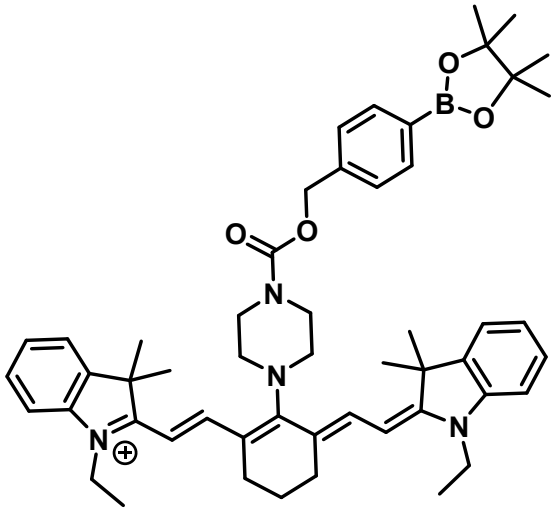
Resorcinol (0.51 g, 4.60 mmol) was added to **Compound 1**[1] (1.15 g, 1.80 mmol) and K_2CO_3 (0.63 g, 4.60 mmol) in MeCN (20 mL). The reaction mixture was stirred at 50 °C for approximately 5 hours (reaction progress followed by TLC). The reaction was diluted with DCM (50 mL) and brine (50 mL) and the organic layer was washed with brine (3 x 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude mixture was purified via silica chromatography (EtOAc to CHCl3/MeOH (95/5)) to afford the title compound as a blue solid (0.63 g, 1.58 mmol, 88 %). ^{1}H NMR (400 MHz, MeOD) δ 8.67 (d, J = 14.5 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.52 – 7.34 (m, 5H), 6.82 (dd, J = 8.6, 2.2 Hz, 1H), 6.78 (d, J = 3.1 Hz, 1H), 6.35 (d, J = 14.5 Hz, 1H), 4.30 (q, J = 7.3 Hz, 2H), 2.78 (t, J = 6.2 Hz, 2H), 2.72 (t, J = 5.9 Hz, 2H), 2.00 – 1.88 (m, 2H), 1.45 (t, J = 7.3 Hz, 3H). Data was consisted with reported literature data.[1]

(*E*)-1-ethyl-3,3-dimethyl-2-(2-(6-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3H-indol-1-ium (HD-BPin)



4-(Bromomethyl)benzeneboronic acid pinacol ester (0.067 g, 0.23 mmol) was added to a solution of **HD-OH** (0.10 g, 0.19 mmol), K_2CO_3 (0.052 g, 0.38 mmol) and KI (0.031 g, 0.019 mmol) in MeCN (2 mL). The reaction mixture was stirred overnight, and the solvent was removed under reduced pressure. The crude mixture was purified via column chromatography (100 % EtOAc to $CHCl_3$/ MeOH (95/5%)) to afford the title compound as a shiny purple solid (0.085 g, 0.14 mmol, 74 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, J = 14.7 Hz, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.54 – 7.45 (m, 4H), 7.46 – 7.38 (m, 2H), 7.36 (s, 1H), 7.19 (s, 1H), 6.97 – 6.90 (m, 1H), 6.86 (d, J = 2.4 Hz, 1H), 6.62 (d, J = 14.9 Hz, 1H), 5.24 (s, 2H), 4.62 (q, J = 7.2 Hz, 2H), 2.85 (t, J = 5.8 Hz, 2H), 2.74 (t, J = 5.7 Hz, 2H), 1.95 (t, J = 5.7 Hz, 2H), 1.78 (s, 6H), 1.54 (t, J = 7.3 Hz, 3H), 1.34 (s, 12H). ^{13}C NMR (151 MHz, CDCl3) δ 176.77, 161.94, 161.56, 154.29, 145.93, 141.89, 141.15, 138.96, 135.27, 133.41, 129.32, 128.77, 127.72, 127.24, 126.62, 122.52, 116.02, 115.35, 113.72, 112.67, 104.33, 102.06, 83.98, 70.93, 50.60, 41.92, 29.25, 28.27, 24.89, 20.31, 13.08. HRMS: m/z calculated for $C_{40}H_{45}BNO_4$: requires 614.3441 for $[M]^+$, found 614.3436.

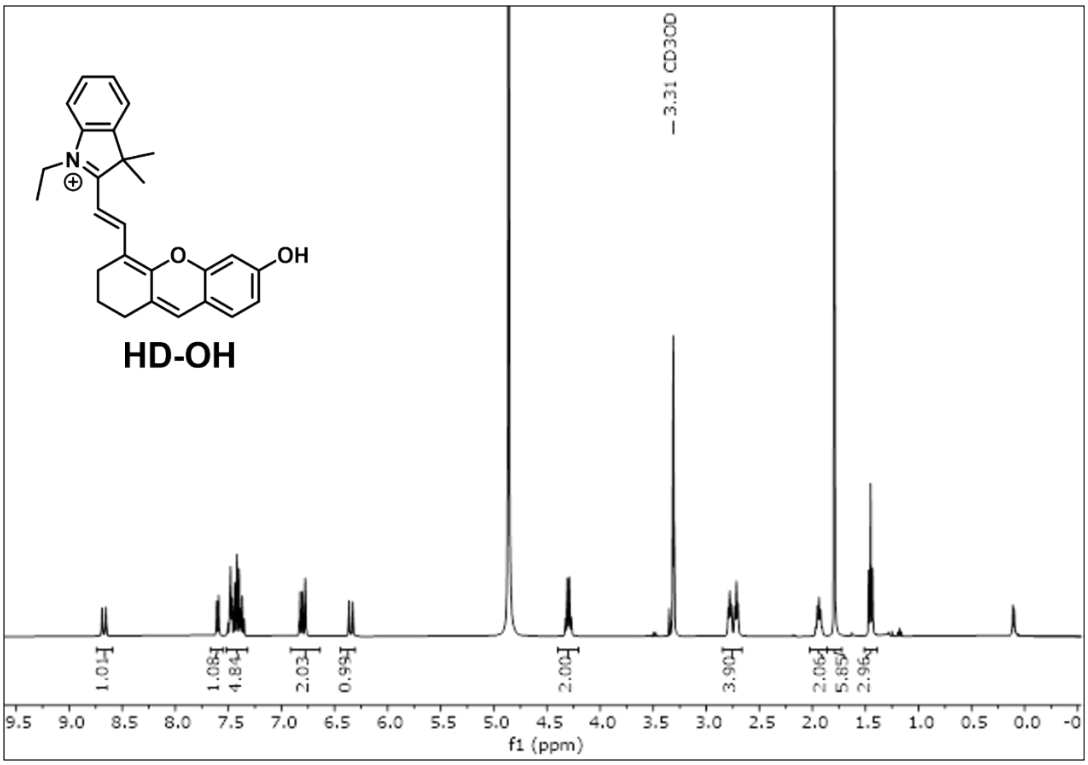
1-Ethyl-2-((*E*)-2-((*E*)-3-(2-((*E*)-1-ethyl-3,3-dimethylindolin-2-ylidene)ethylidene)-2-(4-(((4-(4,4,5,5 tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)piperazin-1-yl)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium (Cy7-BPin)



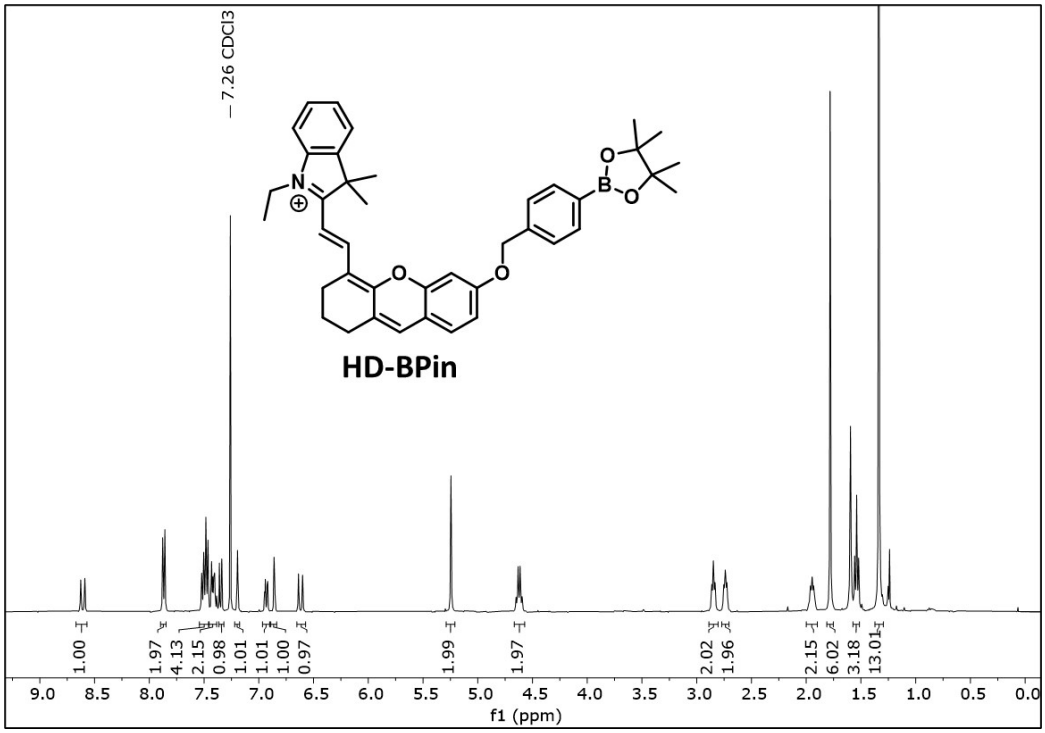
Compound 3[2] (0.17 g, 0.44 mmol) was added to a solution of **Compound 2**[3] (0.20 g, 0.29 mmol) in DCM (3 mL). The reaction mixture was cooled to 0 ^{o}C and DMAP (Catalytic) was added. The reaction was monitored by TLC analysis. Upon completion, the reaction mixture was purified via silica chromatography (5:95, MeOH: $CHCl_3$) to afford the title compound as a dark green solid (0.15 g, 0.18 mmol, 62 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.1 Hz, 2H), 7.70 (d, *J* = 14.1 Hz, 2H), 7.35 (d, *J* = 7.9 Hz, 2H), 7.32 – 7.24 (m, 4H), 7.11 (m, 2H), 7.00 (d, *J* = 7.5 Hz, 2H), 5.94 (d, *J* = 13.5 Hz, 2H), 5.19 (s, 2H), 4.13 – 4.00 (m, 4H), 3.69 (m, 4H), 3.49 (m, 4H), 2.49 (m, 4H), 1.79 (m, 2H), 1.59 (s, 12H), 1.40 – 1.32 (m, 6H), 1.27 (s, 12H); ^{13}C NMR (151 MHz, CDCl3) δ 170.49, 169.41, 155.16, 142.10, 141.68, 140.47, 139.33, 135.08, 128.74, 127.31, 126.55, 124.28, 122.11, 109.85, 97.80, 83.95, 67.97, 67.56, 54.01, 48.43, 39.14, 28.68, 24.87, 12.05; HRMS: m/z calculated for $C_{52}H_{66}BN_4O_4$: requires 821.5179 for $[M]^+$, found 821.5171.

5. NMR Spectra

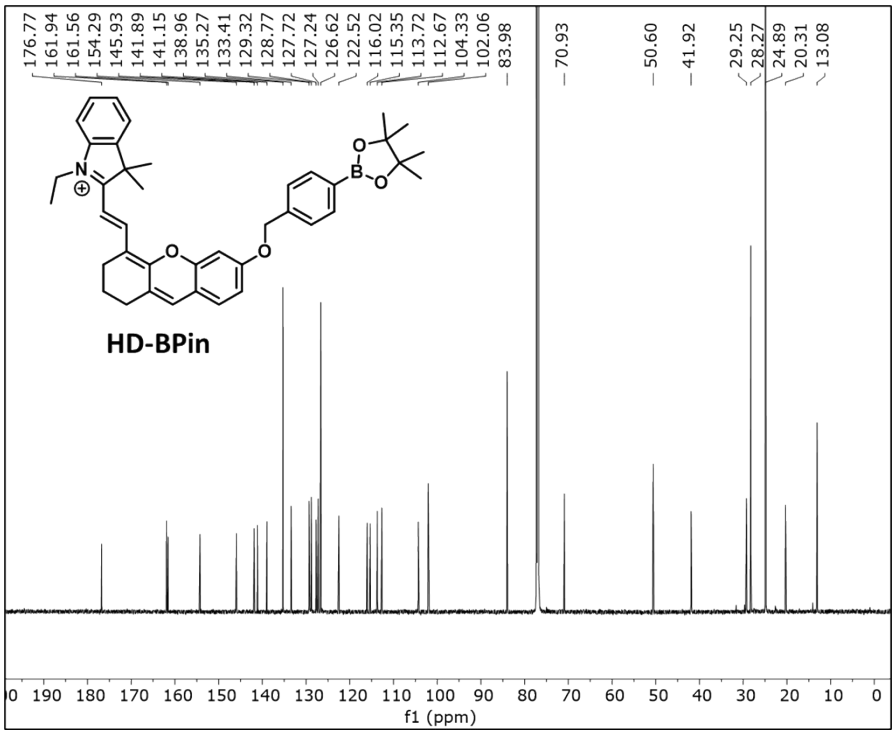
(*E*)-1-ethyl-2-(2-(6-hydroxy-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium (^{1}H NMR, 400 MHz)



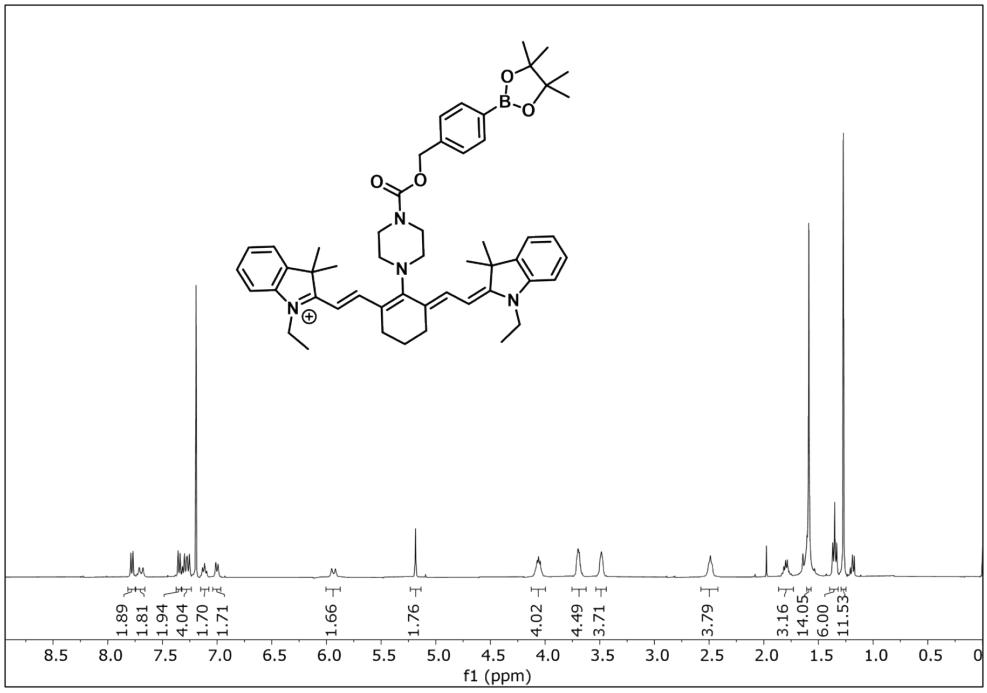
(*E*)-1-ethyl-3,3-dimethyl-2-(2-(6-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3H-indol-1-ium (^{1}H NMR, 400 MHz)



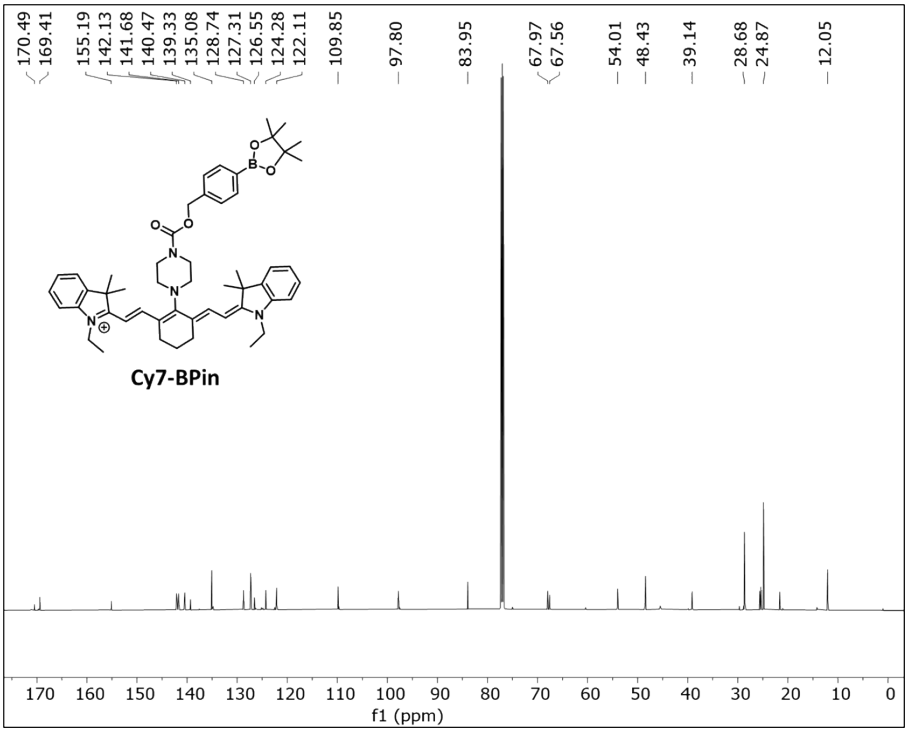
(*E*)-1-ethyl-3,3-dimethyl-2-(2-(6-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3H-indol-1-ium (^{13}C NMR, 151 MHz)



1-Ethyl-2-((*E*)-2-((*E*)-3-(2-((*E*)-1-ethyl-3,3-dimethylindolin-2-ylidene)ethylidene)-2-(4-(((4-(4,4,5,5 tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)piperazin-1-yl)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium (^{1}H NMR, 400 MHz)



1-Ethyl-2-((*E*)-2-((*E*)-3-(2-((*E*)-1-ethyl-3,3-dimethylindolin-2-ylidene)ethylidene)-2-(4-(((4-(4,4,5,5 tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)piperazin-1-yl)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium (^{13}C NMR, 151 MHz). Note – small impurities appeared during long carbon NMR experiment, which suggests potential instability in solution for CY7-BPin.



6. References

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