Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information for

A Modular Trigger for the Development of Selective Superoxide Probes

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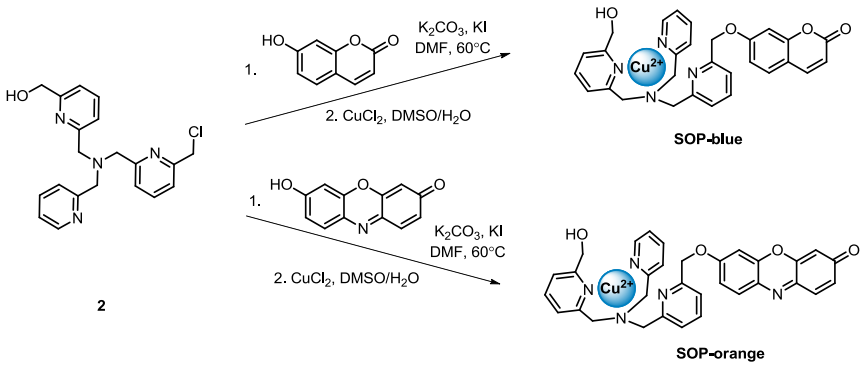
1. Synthesis

General. All solvents were of reagent grade. All commercially available chemicals were used as received. 6-(Hydroxymethyl)-2-pyridinecarboxyaldehyde,[1] 3-(2-benzothiazolyl)-7-hydroxy coumarin,[2] and di-(2-picolyl)amine[3] were synthesized according to literature procedures. LCMS analyses were carried out using a Waters-Alliance e2695 system coupled to a 2489 UV/Vis detector and an ACQUITY QDa MS detector. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were obtained from a Brucker DPX 400 or Brucker DPX 500 spectrometers. Signals were internally referenced to solvent residues. HRMS data was obtained from a Bruker maXis II High Resolution QTOF spectrometer.

Synthesis of 1. A mixture of 2-picolylamine (0.31 ml, 3 mmol) and 6-(hydroxymethyl)-2-pyridinecarbaldehyde (0.42 g, 3 mmol) in 40 ml MeOH was stirred at room temperature for 2 hours. The mixture was cooled in an ice bath and $NaBH_4$ (0.23 g, 6 mmol) was added in small portions. The mixture was slowly warmed to room temperature and stirred for 2 hours. Solvents were removed and the residue was partitioned in CH_2Cl_2 (20 ml) and saturated K_2CO_3 (20 ml). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (20 ml × 2). The organic layers were combined and dried over $MgSO_4$. Solvents were removed by a rotatory evaporator to give **1** which was used in the next step without further purification. Yield = 0.73 g, quant. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 8.56 (d, *J* = 4.5 Hz, 1H), 7.66–7.58 (m, 2H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.25–7.22 (m, 1H), 7.19–7.12 (m, 1H), 7.10 (d, *J* = 7.7 Hz, 1H), 4.74 (s, 2H), 3.99 (s, 2H), 3.97 (s, 2H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298K) δ (ppm) : 159.7, 159.2, 158.1, 149.0, 137.0, 136.5, 122.3, 121.9, 120.5, 118.6, 64.2, 54.4, 54.2. ESI-MS (+ve): calcd. for $C_{13}H_{16}N_3O$ $[M+H]^+$ *m/z* 230.1, found 230.0.

Synthesis of 2. A mixture of **1** (0.45 g, 1.96 mmol), 2,6-dichloromethylpyridine (1.38 g, 7.83 mmol) and $NaHCO_3$ (0.16 g, 1.96 mmol) in 40 ml MeCN was heated at 50°C for overnight. Insoluble materials were removed by filtration. The filtrate was concentrated and purified by a basic alumina column (100% ethyl acetate → 50:1 ethyl acetate/MeOH → 30:1 ethyl acetate/MeOH). The product was obtained as a pale yellow oil. Yield = 0.37 g, 51%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 8.54 (d, *J* = 4.8 Hz, 1H), 7.73–7.52 (m, 5H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.15 (t, *J* = 6.1 Hz, 1H), 7.07 (d, *J* = 7.7 Hz, 1H), 4.73 (s, 2H), 4.65 (s, 2H), 3.89 (s, 6H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298 K) δ 159.2, 159.1, 158.8, 157.7, 155.8, 149.0, 137.5, 137.1, 136.6, 123.1, 122.2, 122.1, 121.6, 121.1, 118.9, 64.1, 60.2, 60.0, 59.7, 46.7. ESI-MS (+ve): calcd. for $C_{20}H_{22}N_4O^{35}Cl$ $[M+H]^+$ *m/z* 369.1, found 369.4; calcd. for $C_{20}H_{22}N_4O^{37}Cl$ $[M+H]^+$ *m/z* 371.1, found 371.3.



Scheme S1. Synthesis of **SOP-blue** and **SOP-orange**.

General procedures for the synthesis of SOP. A mixture of **2**, the fluorescent dye, K_2CO_3 and KI in 40 ml DMF was heated at 60°C for overnight. Insoluble materials were removed by filtration, and the filtrate was purified by a basic alumina column (100% ethyl acetate → 50:1 ethyl acetate/MeOH → 20:1 ethyl acetate/MeOH) to yield the ligand-fluorophore conjugates.

A 2.5 mM stock solution of **SOP** was prepared *in situ* 30 min before use by mixing an equal volume of 5 mM of the purified ligand-fluorophore conjugate in DMSO and 5 mM $CuCl_2$ in deionized water (1:1 mole ratio) in a 1-ml microcentrifuge tube. LCMS analysis of the **SOP** solutions showed both the Cu(II) complexed **SOP** and uncomplexed ligand-fluorophore conjugate in similar ratio even when excess amounts of $CuCl_2$ was used (Figures S2–S4), probably because of slight degree of Cu(II) displacement under the chromatographic conditions.

SOP-cyan. 3-(2-Benzothiazolyl)-7-hydroxycoumarin: 130 mg, 0.44 mmol; **2**: 164 mg, 0.44 mmol; K_2CO_3: 368 mg, 2.64 mmol; KI: 6.6 mg, 0.04 mmol. For the ligand-fluorophore conjugate, yield = 98 mg, 35%. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ 8.96 (s, 1H), 8.53 (d, *J* = 5.1 Hz, 1H), 8.04 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 7.8 Hz, 1H), 7.67–7.53 (m, 5H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.39 (dd, *J* = 7.7, 3.1 Hz, 2H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.26 (s, 1H), 7.15 (t, *J* = 7.4, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 7.03 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 5.28 (s, 2H), 4.73 (s, 2H), 3.93–3.84 (m, 6H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K) δ 163.1, 160.5, 160.2, 159.5, 159.3, 158.5, 157.8, 155.9, 155.1, 152.6, 149.2, 141.7, 137.6, 137.2, 136.7, 136.6, 130.7, 126.5, 125.2, 123.1, 122.8, 122.5, 122.3, 121.8, 121.8, 119.9, 118.9, 117.1, 114.4, 113.2, 102.1, 71.4, 64.1, 60.4, 60.1, 59.7. ESI-MS (+ve): calcd. for $C_{36}H_{30}N_5O_4S$ $[M+H]^+$ *m/z* 628.2, found 628.4. Yield of **SOP-cyan** based on LCMS analysis: 84%. ESI-MS (+ve) **SOP-cyan**: calcd. for $C_{36}H_{29}N_5O_4S^{63}Cu$ $[M]^{2+}$ *m/z* 345.1, found 345.1; calcd. for $C_{36}H_{28}N_5O_4S^{63}Cu$ $[M-H]^+$ *m/z* 689.1, found 689.3. HR-ESI (+ve): calcd. for $C_{36}H_{29}N_5O_4S^{63}Cu$ $[M]^{2+}$ *m/z* 345.0613, found 345.0610; calcd. for $C_{36}H_{28}N_5O_4S^{63}Cu$ $[M-H]^+$ *m/z* 689.1147, found 689.1149.

SOP-blue. 7-Hydroxycoumarin: 36 mg, 0.22 mmol; **2**: 83 mg, 0.22 mmol; K_2CO_3: 182 mg, 1.32 mmol; KI: 0.05 g, 0.22 mmol. For the ligand-fluorophore conjugate: yield = 61 mg, 55%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 8.52 (d, *J* = 5.0 Hz, 1H), 7.71–7.51 (m, 6H), 7.40–7.31 (m, 3H), 7.15 (t, *J* = 7.4 Hz, 1H), 7.09 (d, *J* = 7.7, 1.0 Hz, 1H), 6.92 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.22 (d, *J* = 9.5 Hz, 1H), 5.22 (s, 2H), 4.71 (s, 2H), 3.91–3.85 (m, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$, 298 K) δ 161.7, 161.2, 159.3, 159.2, 158.5, 157.8,

155.8, 155.4, 149.0, 143.4, 137.5, 137.3, 136.8, 129.0, 123.2, 122.3, 121.8, 119.8, 119.0, 113.4, 113.0, 102.4, 71.2, 64.0, 60.1, 60.1, 59.6. ESI-MS (+ve): calcd. for $C_{29}H_{27}N_4O_4$ $[M+H]^+$ *m/z* 495.2, found 495.2. Yield of **SOP-blue** based on LCMS analysis: 76%. ESI-MS (+ve) **SOP-blue**: calcd. for $C_{29}H_{26}N_4O_4{}^{63}Cu$ $[M]^{2+}$ *m/z* 278.6, found 279.0; calcd. for $C_{29}H_{25}N_4O_4{}^{63}Cu$ $[M-H]^+$ *m/z* 556.1, found 556.1. HR-ESI (+ve): calcd. for $C_{29}H_{26}N_4O_4{}^{63}Cu$ $[M]^{2+}$ *m/z* 278.5619, found 278.5620; calcd. for $C_{29}H_{25}N_4O_4{}^{63}Cu$ $[M-H]^+$ *m/z* 556.1161, found 556.1167.

3-orange. Resorufin sodium salt: 116 mg, 0.49 mmol; **2**: 183 mg, 0.49 mmol; K_2CO_3: 205 mg, 1.47 mmol; KI: 6.6 mg, 0.04 mmol. For the ligand-fluorophore conjugate, yield = 107 mg, 40%. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ 8.51 (d, *J* = 4.9 Hz, 1H), 7.74–7.58 (m, 5H), 7.53 (dd, *J* = 7.7, 4.8 Hz, 2H), 7.41–7.30 (m, 3H), 7.18–7.07 (m, 2H), 7.01 (dd, *J* = 8.9, 2.7 Hz, 1H), 6.89 (d, *J* = 2.6 Hz, 1H), 6.79 (dd, *J* = 9.8, 2.0 Hz, 1H), 6.22 (d, *J* = 2.1 Hz, 1H), 5.27 (s, 2H), 4.71 (s, 2H), 3.93–3.84 (m, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$, 298K) δ 186.3, 162.4, 159.2, 159.0, 158.6, 157.6, 155.0, 149.8, 149.1, 145.7, 145.5, 137.5, 137.2, 136.6, 134.7, 134.2, 131.6, 128.6, 123.1, 122.6, 122.2, 121.7, 119.9, 118.9, 114.2, 110.0, 106.7, 101.4, 71.5, 64.0, 60.0, 59.6. ESI-MS (+ve): calcd. for $[M+H]^+$ $C_{32}H_{28}N_4O_4$ *m/z* 546.2, found 546.2. Yield of **SOP-orange** based on LCMS analysis: 72%. ESI-MS (+ve) **SOP-orange**: calcd. for $C_{32}H_{27}N_5O_4{}^{63}Cu$ $[M]^{2+}$ *m/z* 304.1, found 304.0; calcd. for $C_{32}H_{26}N_5O_4{}^{63}Cu$ $[M-H]^+$ *m/z* 607.1, found 607.3. HR-ESI (+ve): calcd. for $C_{32}H_{27}N_5O_4{}^{63}Cu$ $[M]^{2+}$ *m/z* 304.0674, found 304.0671; calcd. for $C_{32}H_{26}N_5O_4{}^{63}Cu$ $[M-H]^+$ *m/z* 607.1270, found 607.1271.

2. Fluorescent Spectroscopy

Fluorescence Spectra were recorded on an Edinburgh Instruments FS5 Spectrofluorometer equipped with a 150 W CW Ozone-free xenon arc lamp and a Photomultiplier R928P detection unit with spectral coverage of 200 nm–870 nm. Samples for emission measurement were contained in a quartz cuvette with a path length of 1 cm and 1.5 ml cell volume. Millipore water was used to prepare all aqueous solutions.

For emission measurement with superoxide, a **SOP** probe solution was prepared by adding 2 µl of the 2.5 mM **SOP** stock to 50 mM Tris (pH 7.6) buffer, followed by 30 µL of 2.5 mM hypoxanthine (HX) and 10 µl of 1000 mU/ml xanthine oxidase (XOD) to give a solution of a final volume of 1000 µl, with the **SOP**, HX, XOD at final concentrations of 5 µM, 75 µM and 10 mU/ml respectively. If KO_2 was used, 50 µl of 1 mM KO_2 in DMSO was added to the **SOP** solution in buffer to give a solution of a final volume of 1000 µl, with the **SOP** and KO_2 at final concentrations of 5 µM and 50 µM respectively. For emission measurement in the presence of TEMPOL or SOD, 100 µl of TEMPOL or superoxide dismutase (4000 U/ml) was added to the **SOP** solution in buffer to give a solution of a final concentration of 4 mM of TEMPOL or 400 U/ml of the SOD. For selectivity studies of **SOP** towards other reactive species, 20 eq (100 µM) of H_2O_2, ^tBuOOH, ClO^-, $^{\cdot}OH$, $^1O_2$, $^{\cdot}NO$, $ONOO^-$ and H_2S, either from commercial source or generated according to literature procedures,[4-6] was added to the **SOP** solutions in buffer from their stock solutions, and the emission spectra were collected after reaction for 1 hour. For emission measurement of with GSH, 40 µl of 50 mM GSH in the Tris buffer was added. For emission measurement in the presence of other transition metals and amino acids, 1 µl of 1 mM stock solutions of Fe^{2+}, Fe^{3+} or Zn^{2+}, or 2 µl of 50 mM stock solutions of the tested amino acids was added to the **SOP** solution in the buffer to give a solution of a final volume of 1000 µl, with the **SOP**, metal ions or amino acids at final concentrations of 5 µM, 1 µM and 100 µM respectively. For emission measurement in the presence of Cys or GSH, 4 mM TEMPOL was added to scavenge superoxide produced from air oxidation of the thiols.

The solutions were mixed well in the quartz cuvette with a plastic disposable pipet after addition of each reagent and at 10 min intervals prior to emission spectrum to be recorded. **SOP-cyan**, **SOP-blue** and **SOP-orange** were excited at 455 nm, 325 nm and 570 nm respectively and the corresponding emission spectra were collected from 465–650 nm, 335–600 nm and 575–700 nm respectively.

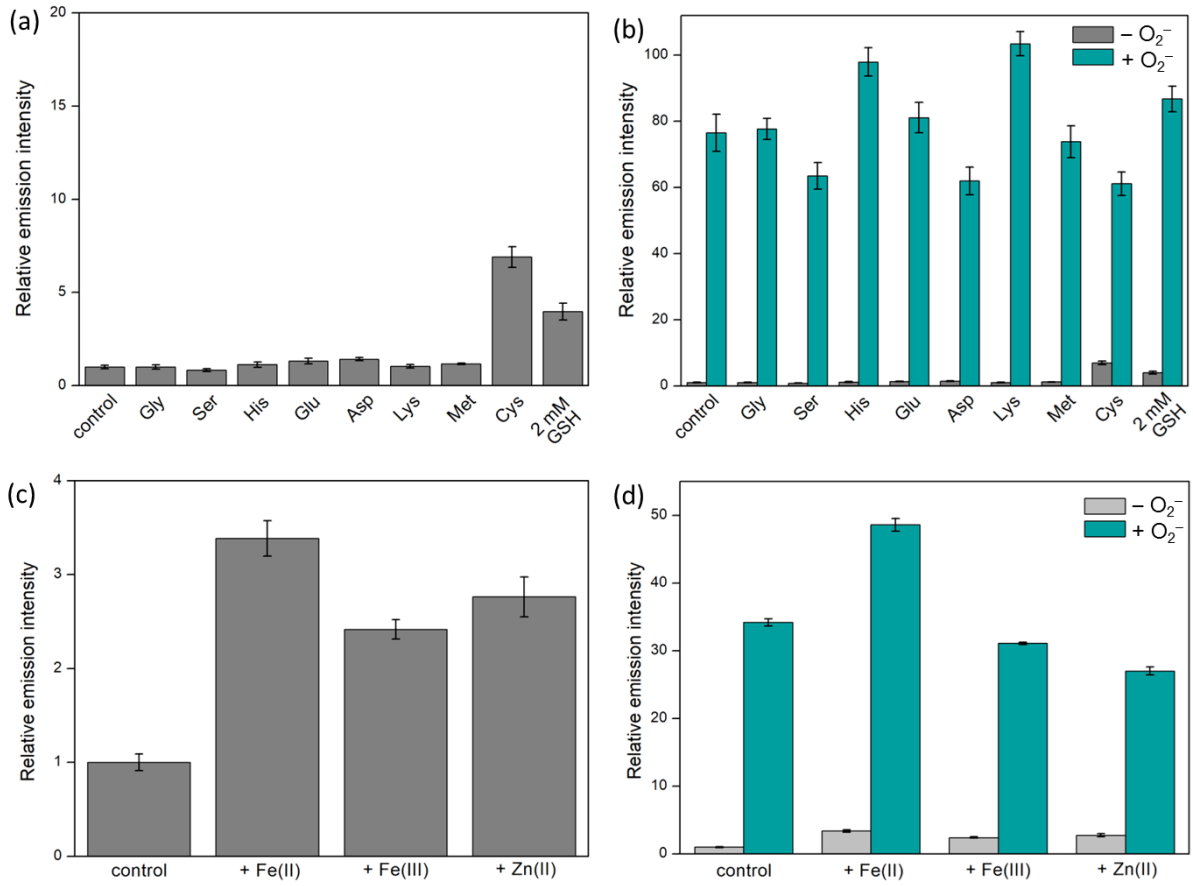


Figure S1. Fluorescent response of 5 μM of **SOP-cyan** towards (a) 100 μM of amino acids or 2 mM GSH; (b) superoxide in the presence of 100 μM of amino acids or 2 mM GSH; (c) 5 μM of Fe^{2+}, Fe^{3+} and Zn^{2+}; and (d) superoxide in the presence of 5 μM of Fe^{2+}, Fe^{3+} and Zn^{2+}. λ_{ex} = 455 nm. KO_2 was used in the measurement with other transition metal ions as Zn^{2+} is known to inhibit XOD activity. Error bars are ±SD (n = 3).

3. LCMS analysis

LCMS analyses were carried out using a Waters-Alliance e2695 system coupled to a 2489 UV/Vis detector and an ACQUITY QDa MS detector. Samples for LCMS analysis were prepared as follows: 4 µl of a 2.5 mM **SOP** stock was added to 50 mM Tris (pH 7.6) buffer, followed by addition of 60 µl of a 2.5 mM HX solution and 20 µl of a 1000 mU/ml XOD to give a solution of a final volume of 200 µl, with concentrations of the **SOP**, HX and XOD at 50 µM, 750 µM and 100 mU/ml respectively. The mixture was allowed to react for 1 hour, which was then injected onto a LC-MS (Waters Alliance e2695 with an ACQUITY QDa Mass Detector) with a SunFire C18 3.5 µm 3.0 × 150 mm column eluted at 25ºC, 0.6 ml/min flow rate, using a gradient of CH_3CN/water (with 0.5% formic acid) from 5% to 100% over 25 min. Absorption was monitored at 350 nm. Xanthine oxidase (from bovine milk, lyophilized powder) and superoxide dismutase (from bovine erythrocytes, lyophilized powder) were purchased from Sigma-Aldrich and were dissolved in 50 mM Tris buffer at pH 7.6 at the required concentrations before use. For cleavage study of **SOP-cyan** at different pH, 50 mM phosphate buffers at pH 4.5, 5.5, 6.5, 7.5 and 8.5 were used to prepare the solutions. Concentration of the cleaved 3-(2-benzothiazolyl)-7-hydroxycoumarin was calculated from the area of the corresponding peak in the chromatograms against a calibration curve constructed from stock solutions (1–5 µM) of 3-(2-benzothiazolyl)-7-hydroxycoumarin.

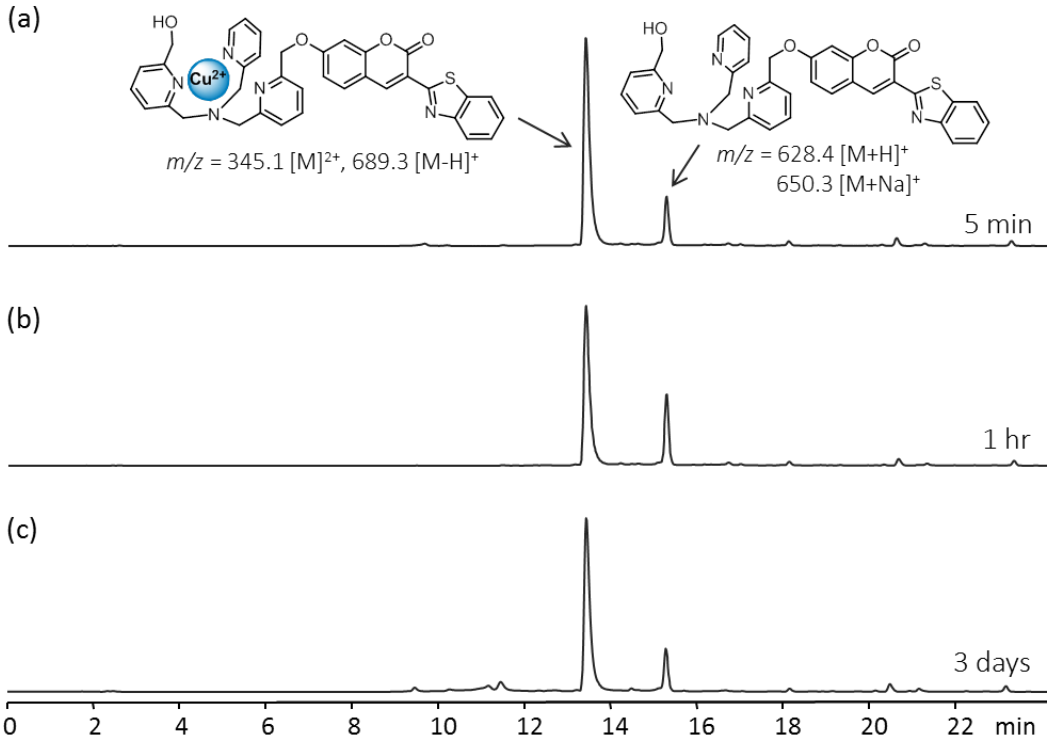


Figure S2. LCMS analysis of a 200 µM solution of **SOP-cyan** after (a) 5 min; (b) 1 hour and (c) 3 days of preparation. A slight degree of Cu(II) displacement from the probe under the chromatographic condition was observed. UV absorbance was monitored at 350 nm.

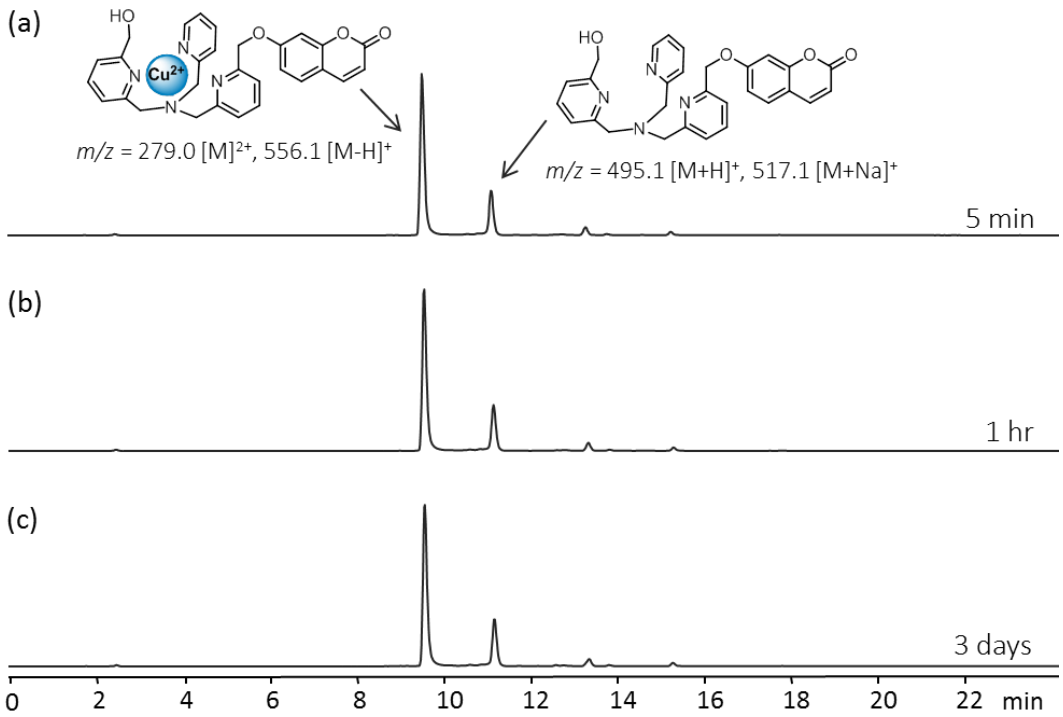


Figure S3. LCMS analysis of a 200 μM solution of **SOP-blue** after (a) 5 min; (b) 1 hour and (c) 3 days of preparation. UV absorbance was monitored at 350 nm.

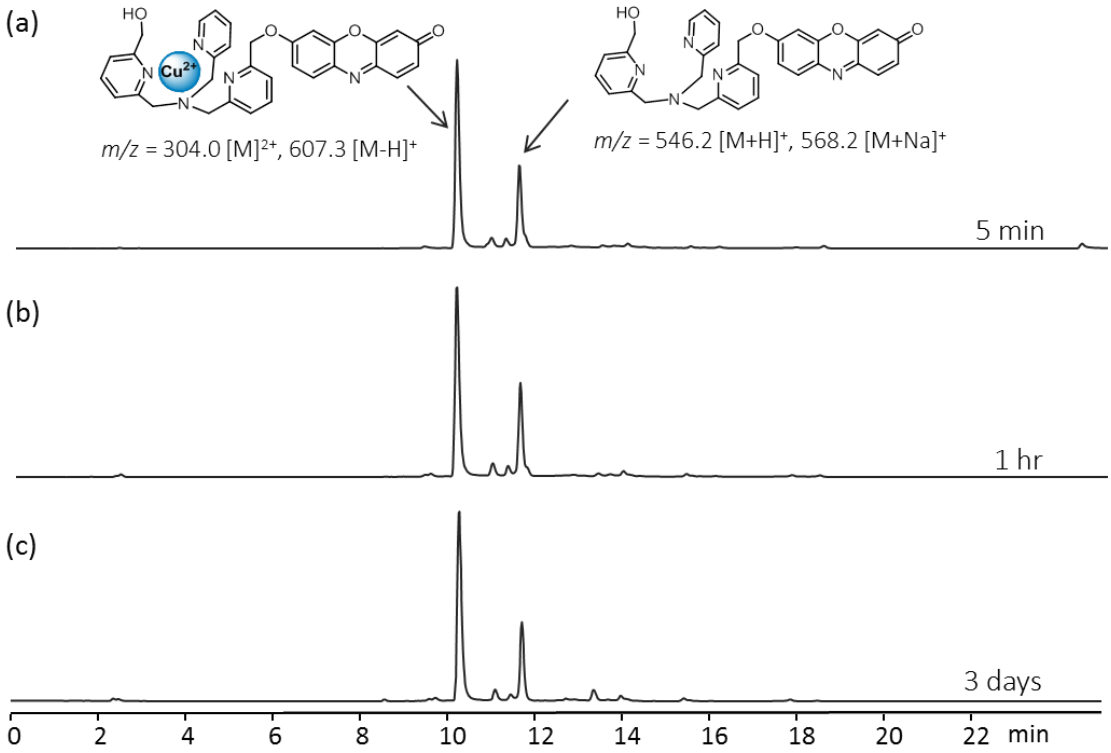


Figure S4. LCMS analysis of a 200 μM solution of **SOP-orange** after (a) 5 min; (b) 1 hour and (c) 3 days of preparation. UV absorbance was monitored at 350 nm.

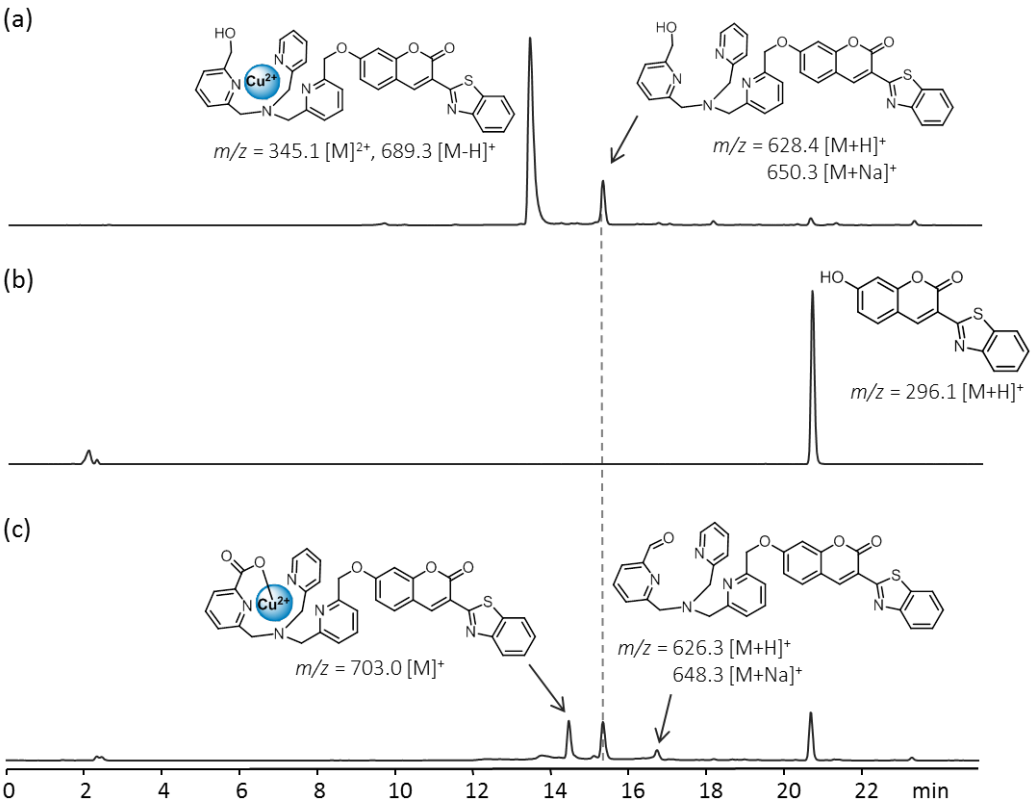


Figure S5. LCMS analysis of (a) **SOP-blue**; (b) 7-hydroxycoumarin; and (c) **SOP-blue** after reaction with superoxide from XOD/HX for 1 hour. UV absorbance was monitored at 350 nm.

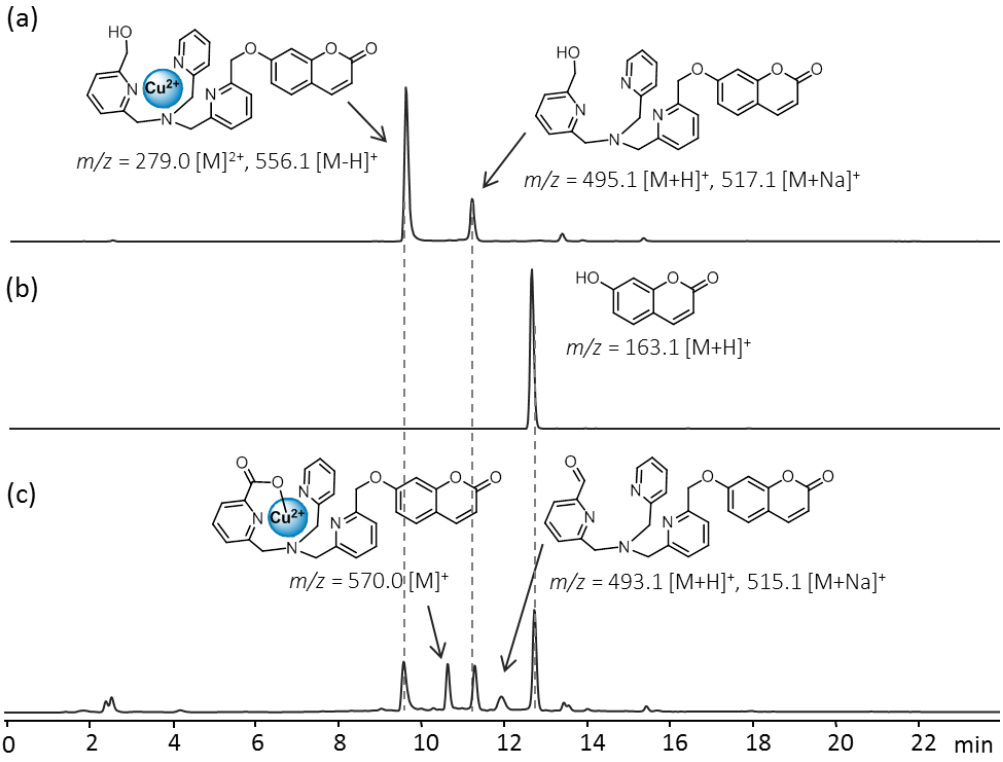


Figure S6. LCMS analysis of (a) **SOP-cyan**; (b) 3-(2-benzothiazolyl)-7-hydroxycoumarin; and (c) **SOP-cyan** after reaction with superoxide from XOD/HX for 1 hour. UV absorbance was monitored at 350 nm.

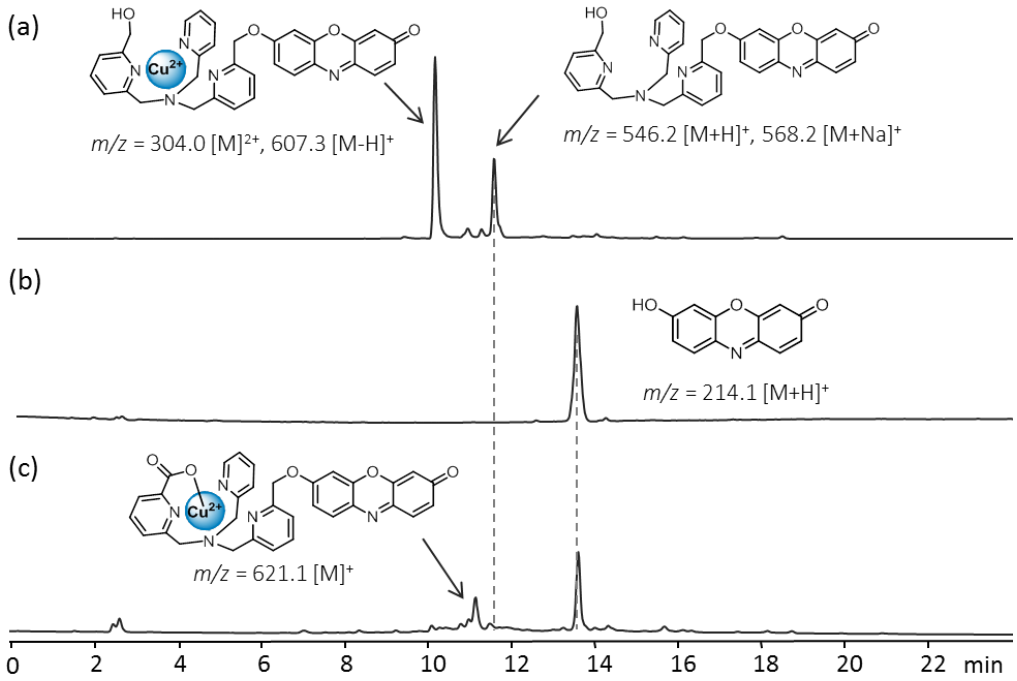


Figure S7. LCMS analysis of (a) **SOP-orange**; (b) resorufin; and (c) **SOP-orange** after reaction with superoxide from XOD/HX for 1 hour. UV absorbance was monitored at 350 nm.

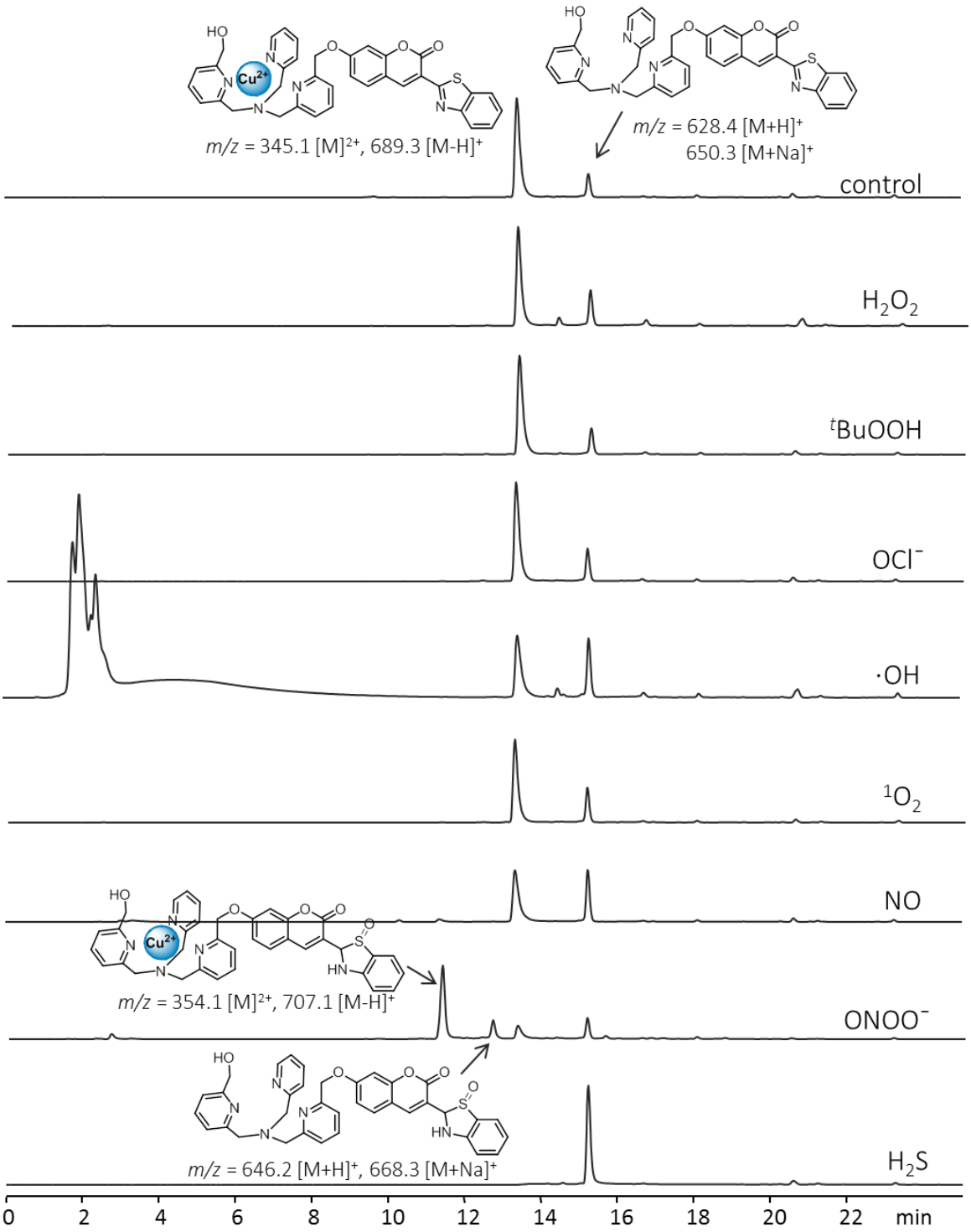


Figure S8. LCMS analysis of 200 μM **SOP-cyan** after reaction with 4 mM of various reactive species for 1 hour. UV absorbance was monitored at 350 nm. For the reaction of **SOP-cyan** with peroxynitrite, products from the oxidation at the benzothiazole part (retention time = 11.4 and 12.8 min) were observed. Similar products have also been identified from the reaction of 3-(2-benzothiazolyl)-7-hydroxycoumarin with peroxynitrite under the same condition.

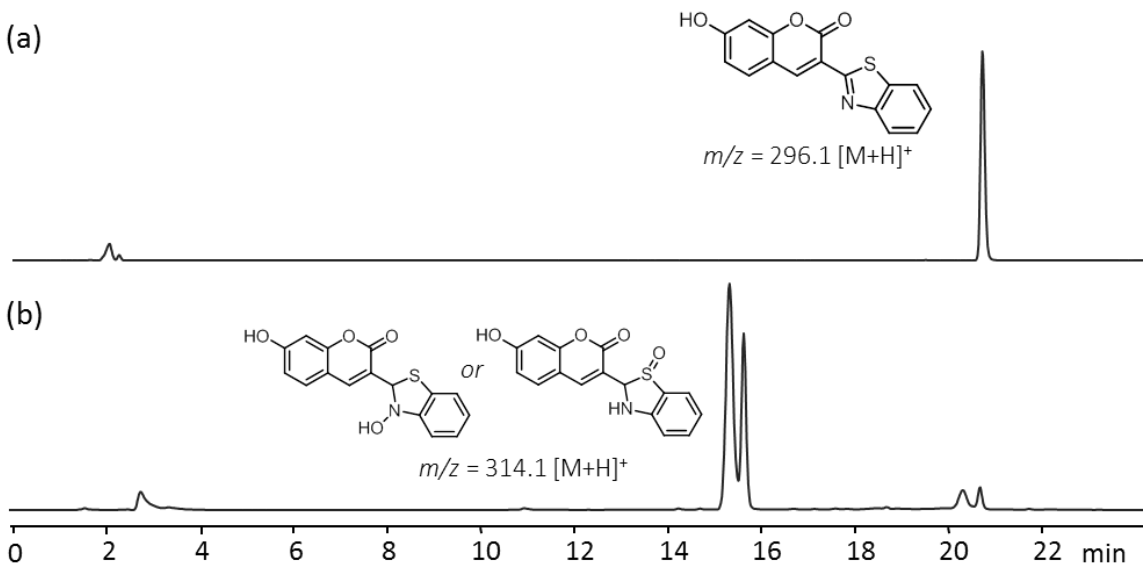


Figure S9. LCMS analysis of (a) 50 μM 3-(2-benzothiazolyl)-7-hydroxycoumarin and (b) the reaction of 50 μM 3-(2-benzothiazolyl)-7-hydroxycoumarin with 1 mM of peroxynitrite for 1 hour. UV absorbance was monitored at 350 nm.

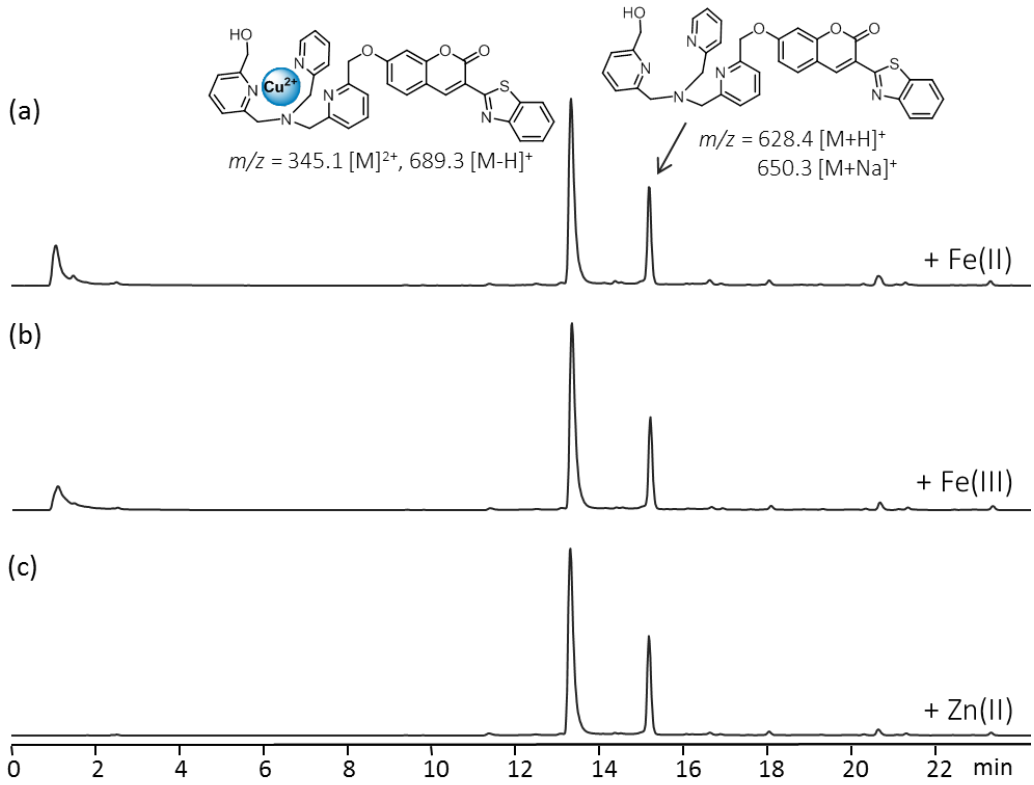


Figure S10. LCMS analysis of a mixture of 200 μM **SOP-cyan** with one equivalent of (a) Fe^{2+}; (b) Fe^{3+}; and (c) Zn^{2+}. UV absorbance was monitored at 350 nm.

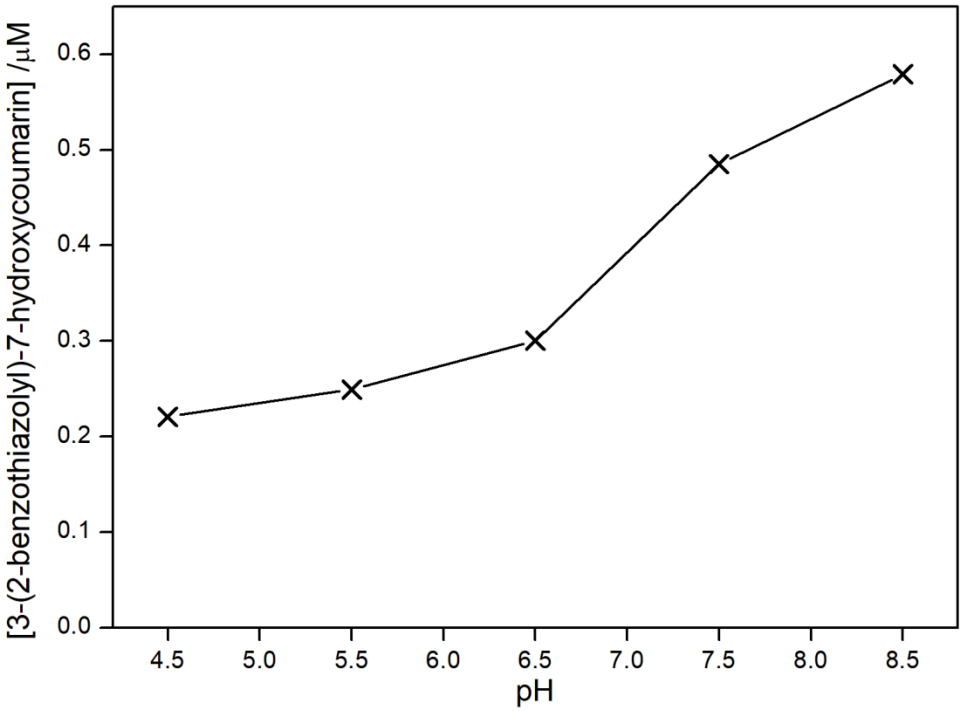


Figure S11. Concentration of released 3-(2-benzothiazolyl)-7-hydroxycoumarin from reactions of **SOP-cyan** with XOD/HX at different pH as determined by the peak area from the respective chromatograms. The difference in the cleavage yield at different pH is ascribed to the difference in XOD activity at different pH.[7]

4. Live Cell Imaging

Cells were grown in the UC Berkeley Tissue Culturing Facility. Human cervical epithelial carcinoma (HeLa) and human epidermoid carcinoma (A431) were maintained in Dulbecco's Modified Eagle Medium (DMEM, high glucose; Invitrogen) supplemented with GlutaMAX (Gibco) and 10 vol% fetal bovine serum (FBS). HEK293T cells were maintained in DMEM (high glucose) supplemented with GlutaMAX, 10 vol% FBS and 1 vol% non-essential amino acids (NEAA, Gibco). All cells were incubated at 37^{o}C in 5% CO_2 humidified air, and subcultured when 80% confluence was reached. For cell-imaging experiments, the cells were plated on 8-well Lab Tek borosilicate chambered coverglass slides (Nunc), and allowed to grow to *ca*. 70% confluency before performing the cell imaging experiments.

Confocal fluorescence cell imaging was performed with a Zeiss laser scanning microscope 710 with 20× objective lens or 63× oil-immersion objective lens using Zen 2009 software (Carl Zeiss). **SOP-cyan** was excited at 458 nm and its emission was collected between 465 and 709 nm. DRAQ5 was excited at 633 nm with a HeNe laser and its emission was collected between 661 and 759 nm. For co-localization experiment, the cells were incubated with LysoTracker Red DND-99 (75 nM), ER-Tracker Red (1 μM) or MitoTracker Deep Red FM (100 nM) in the culture medium for 30 min, and the dyes were excited at 543 nm, 594 nm, and 633 nm respectively, with their emission collected between 566 nm–690 nm, 599 nm–734 nm and 640 nm–758 nm resp For imaging of superoxide production in cells stimulated by menadione, HeLa or HEK293T cells were grown to *ca.* 70% confluency, incubated with 5 μM **SOP-cyan** and 10 μM menadione in Dulbecco's phosphate-buffered saline (DPBS, with calcium and magnesium) for 30 min at 37^{o}C in 5% CO_2 humidified air. At the last 5-min incubation, a nucleus stain, DRAQ5 (5 μM), was added to the culture medium and the cells were maintained at 37^{o}C in 5% CO_2 humidified. The cells were imaged by confocal fluorescence microscopy without further washing.

For imaging of inhibition of superoxide production in cells treated with PEG-SOD, HeLa and HEK293T cells were grown to *ca.* 40% confluency and incubated with PEG-SOD (40 U/ml) in the culture medium for 24 hours. The medium was then changed to solution mixture containing **SOP-cyan** (5 μM) and PEG-SOD (40 U/ml) in DPBS, and the cells were maintained at 37^{o}C in 5% CO_2 humidified air for 30 min. At the last 5-min incubation, DRAQ5 (5 μM) was added to the culture medium. The cells were imaged by confocal fluorescence microscopy without further washing.

For imaging of changes in endogenous superoxide production in A431 cells treated with EGF or DPI, the cells were grown to *ca.* 70% confluency and were incubated with EGF (100 ng/ml) or DPI (5 μM) in the culture medium for 2 hours. The medium was then changed to solution containing EGF (100 ng/ml) or DPI (5 μM) and **SOP-cyan** (5 μM), in DPBS, and the cells were maintained at 37^{o}C in 5% CO_2 humidified air for 30 min. At the last 5-min incubation, DRAQ5 (5 μM) was added to the culture medium. The cells were imaged by confocal fluorescence microscopy without further washing.

The average fluorescence intensity from cells was determined using ImageJ, by setting a threshold value for selection of a region of interest (ROI) in the image and measuring the mean fluorescence intensity in the ROI. All experiments were performed in triplicate, and statistical analyses were performed with a two-tailed Student's *t*-test.

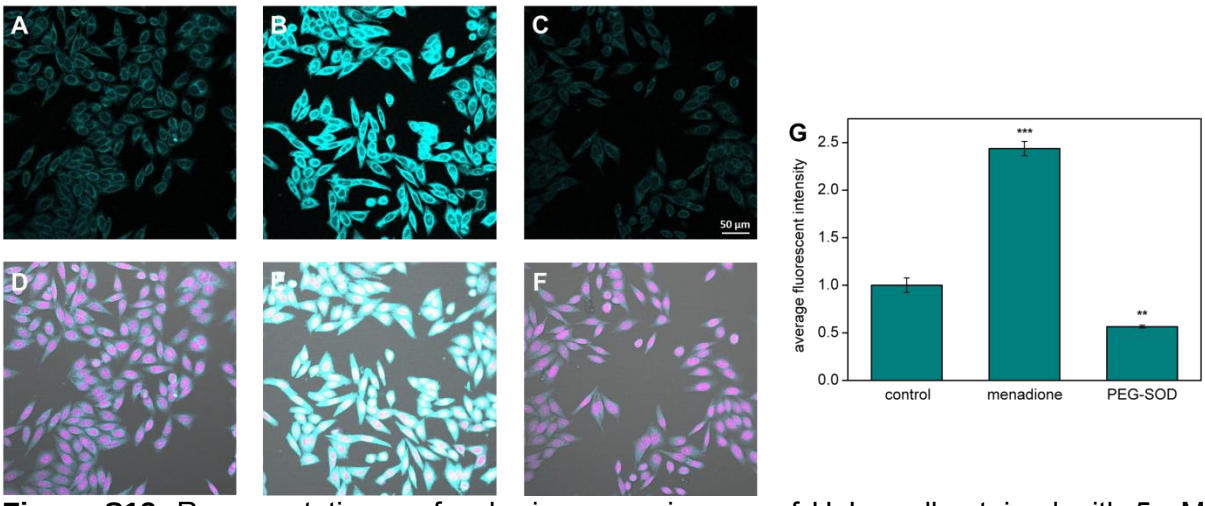


Figure S12. Representative confocal microscopy images of HeLa cells stained with 5 µM **SOP-cyan** for 30 min. (A) Control cells. (B) Cells treated with 10 µM menadione for 30 min. (C) Cells treated with PEG-SOD (40 U/ml) for 24 hours. (D–F) Overlays of bright field, **SOP-cyan** and far-red nuclear stain DRAQ5^{TM} images in A–C. (G) Average fluorescent intensity of **SOP-cyan** in HeLa cells treated under the conditions in A–C from triplicate experiments. Error bars are ±SD (n = 3). Statistical analysis was performed with a two-tailed Student's *t* test. **: $p < 0.01$, ***: $p < 0.001$. Scale bar = 50 µm.

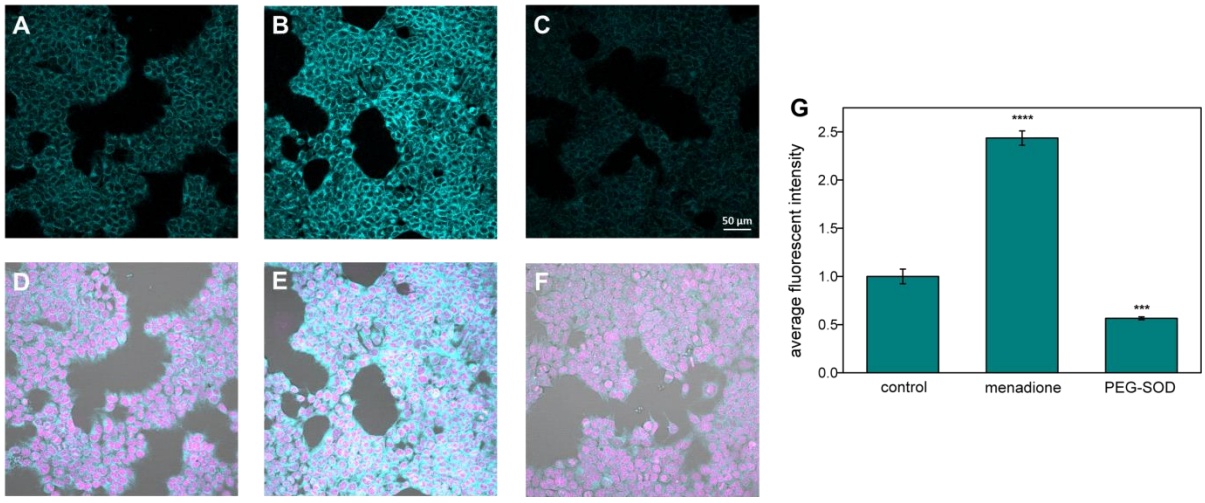


Figure S13. Representative confocal microscopy images of HEK293T cells stained with 5 µM **SOP-cyan** for 30 min. (A) Control cells. (B) Cells treated with 10 µM menadione for 30 min. (C) Cells treated with PEG-SOD (40 U/ml) for 24 hours. (D–F) Overlays of bright field, **SOP-cyan** and far-red nuclear stain DRAQ5^{TM} images in A–C. (G) Average fluorescent intensity of **SOP-cyan** in HEK293T cells treated under the conditions in A–C from triplicate experiments. Error bars are ±SD (n = 3). Statistical analysis was performed with a two-tailed Student's *t* test. ****: $p < 0.0001$, ***: $p < 0.001$. Scale bar = 50 µm.

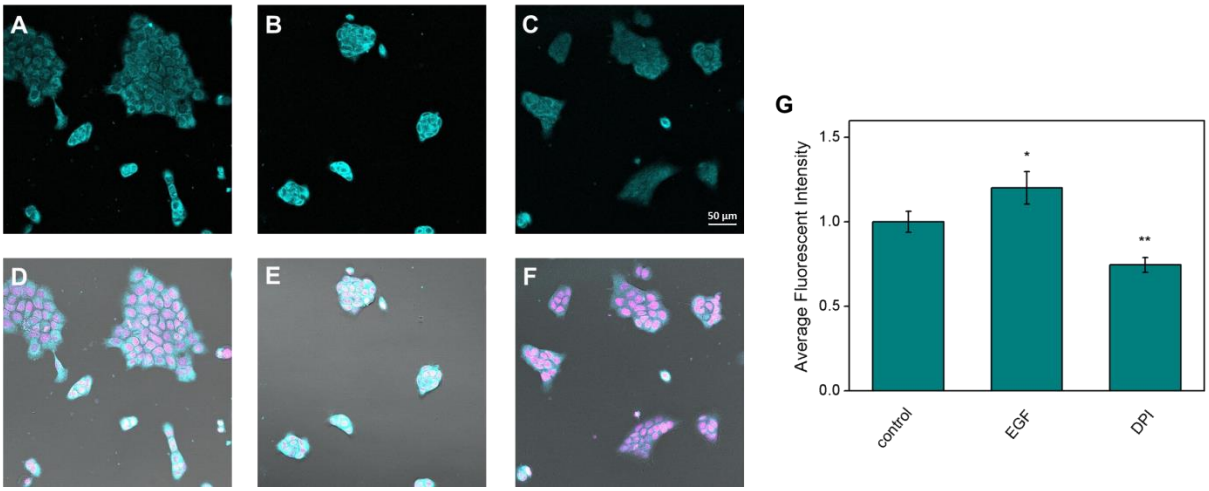


Figure S14. Representative confocal microscopy images of A431 cells stained with 5 µM **SOP-cyan** for 30 min. (A) Control cells. (B) Cells treated with EGF (100 ng/ml) for 4.5 hours. (C) Cells treated with 5 µM DPI for 4.5 hours. (D–F) Overlays of bright field, **SOP-cyan** and far-red nuclear stain DRAQ5™ images in A–C. (G). Average fluorescent intensity of **SOP-cyan** in A431 cells treated under the conditions in A–C from triplicate experiments. Error bars are ±SD (n = 3). Statistical analysis was performed with a two-tailed Student's *t* test. *: $p < 0.05$, **: $p < 0.01$. Scale bar = 50 µm.

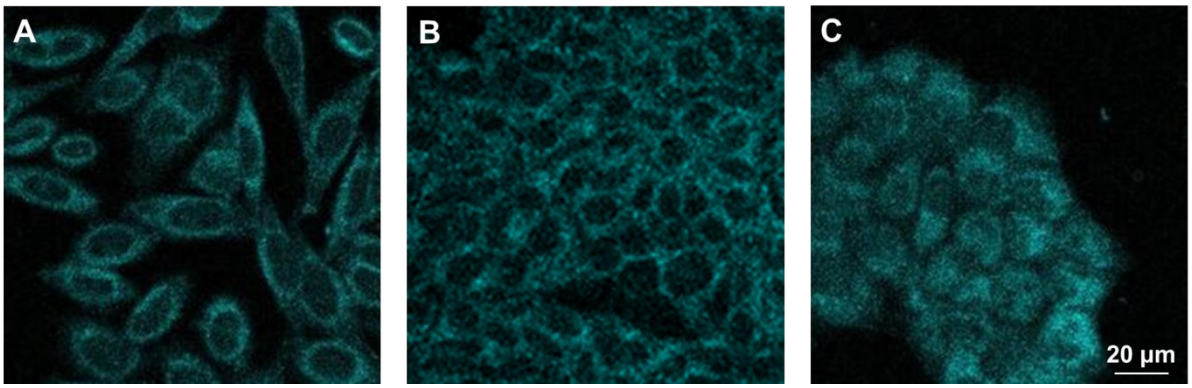


Figure S15. Representative confocal microscopy images of (A) HeLa, (B) HEK293T and (C) A431 cells stained with 5 µM **SOP-cyan** for 30 min at larger magnification. Scale bar = 20 µm.

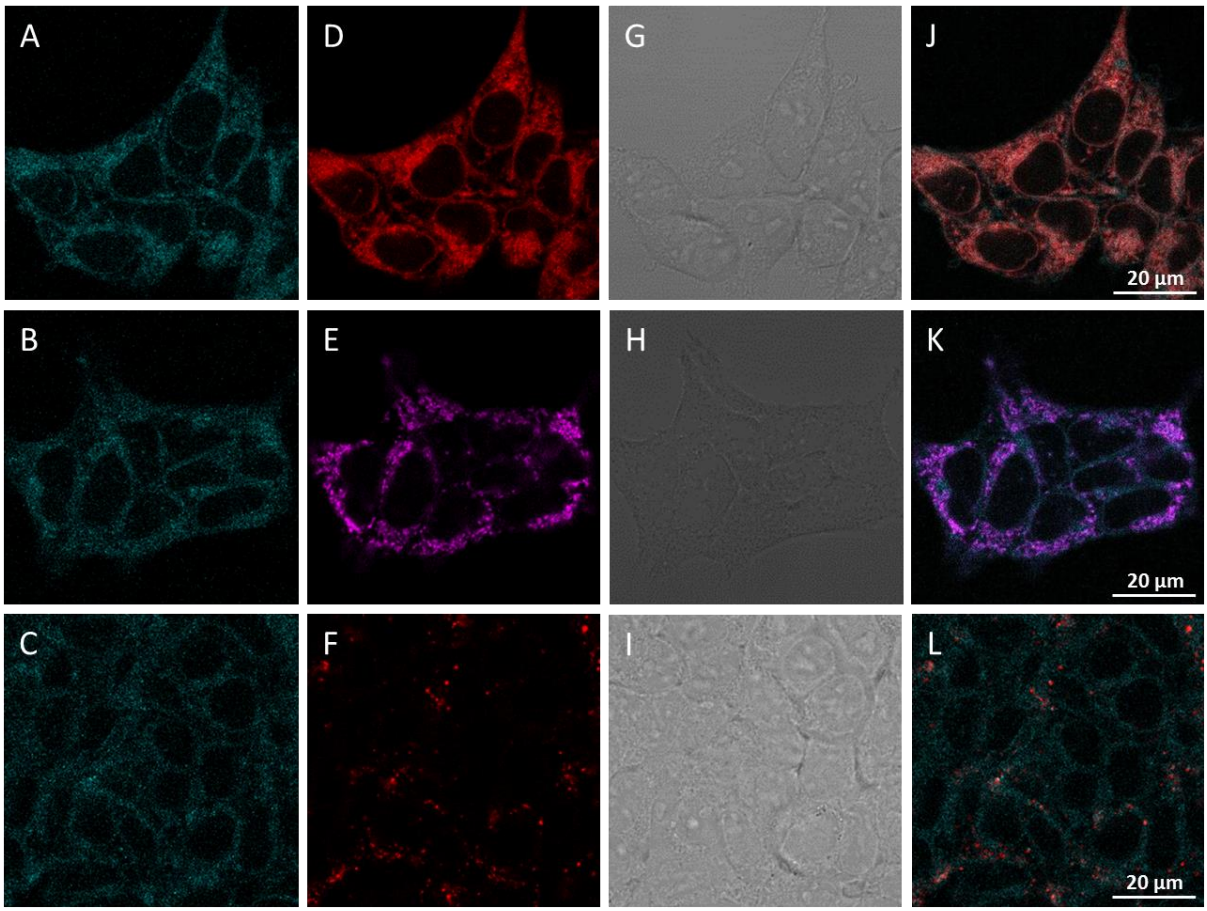


Figure S16. Representative confocal microscopy images of HEK293T cells stained with (A–C) 5 µM **SOP-cyan**; (D) 1 µM ER-Tracker Red; (E) 100 nM MitoTracker Deep Red FM; and (F) 75 nM LysoTracker Red DND-99. (G–I) Bright field imaging of cells in A–F. (J–L) Overlays of **SOP-cyan** and organelle tracker images in A–F. Scale bar = 20 µm.

5. MTT assay

HeLa cells were cultured in Minimum Essential Media (MEM) supplemented with 10 vol% fetal bovine serum (FBS), 1 vol% antibiotic PS and 1 vol% sodium pyruvate. The cells were incubated at 37 ºC in 5% CO_2. One day before the experiment, the cells were passaged and plated on clear-bottom, 96-well plates (10,000 cells/well) and incubated for overnight at 37 ºC in 5% CO_2. The cells were incubated with various concentration of **SOP** for 2 hours and then the MTT reagent (Thermo Fisher, 10 µl/well) was added to cells and incubated for an additional 4 hours at 37 ºC. An SDS-HCl solution (100 µl/well) was added to the cells and incubated for 12 hours at 37 ºC. Absorbance was measured at 570 nm using a plate reader.

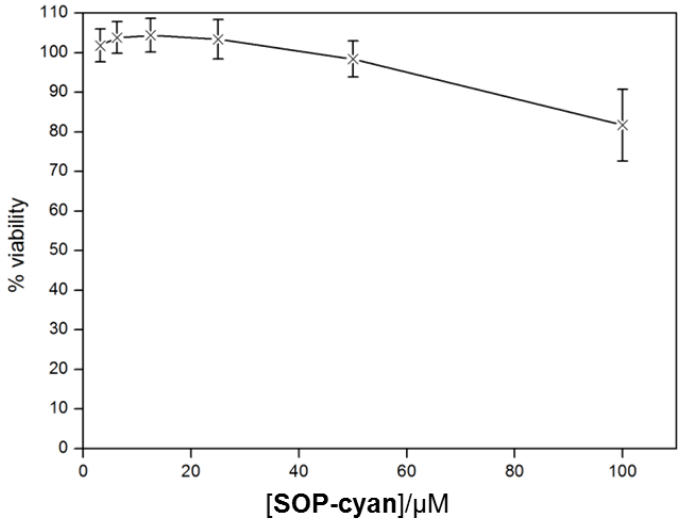


Figure S17. Percentage cell viability of HeLa cells incubated at different concentrations of **SOP-cyan** for 2 hours as determined by MTT assays. Error bars are ±SD (n = 6).

6. NMR spectra

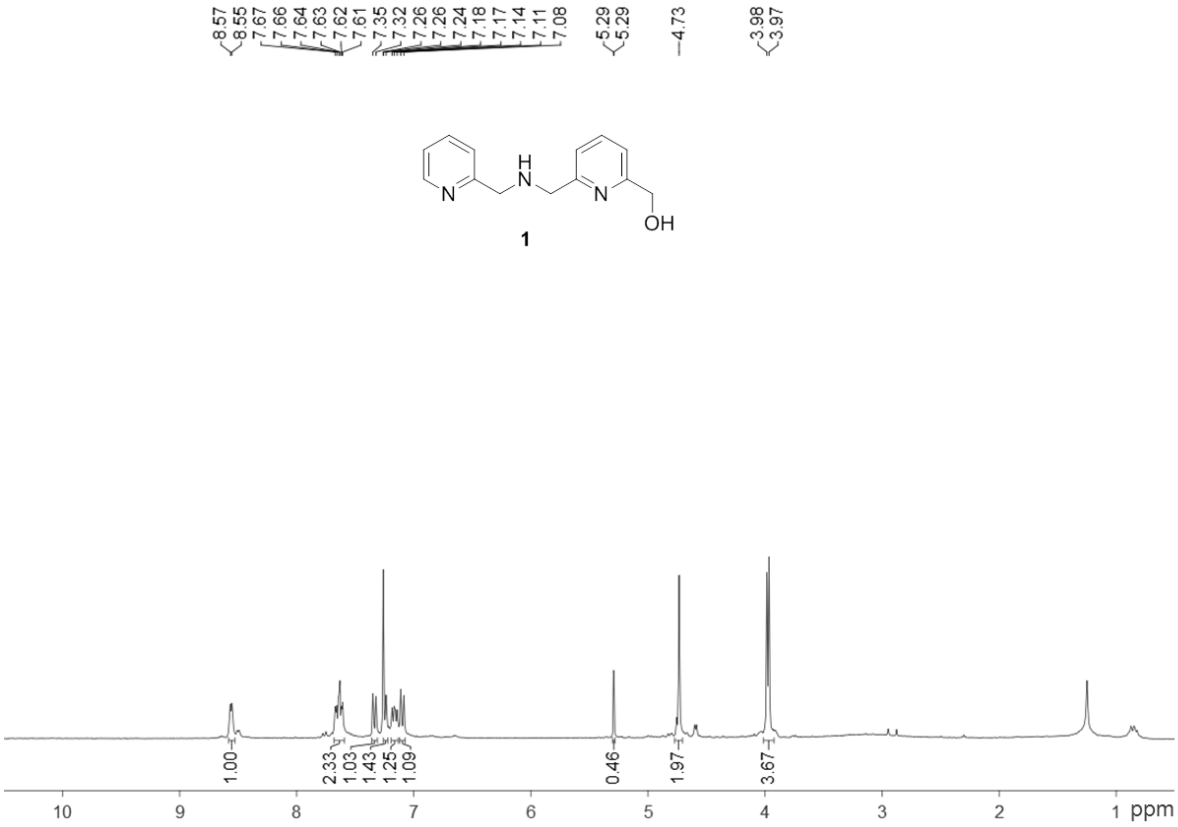


Figure S18. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) of **1**.

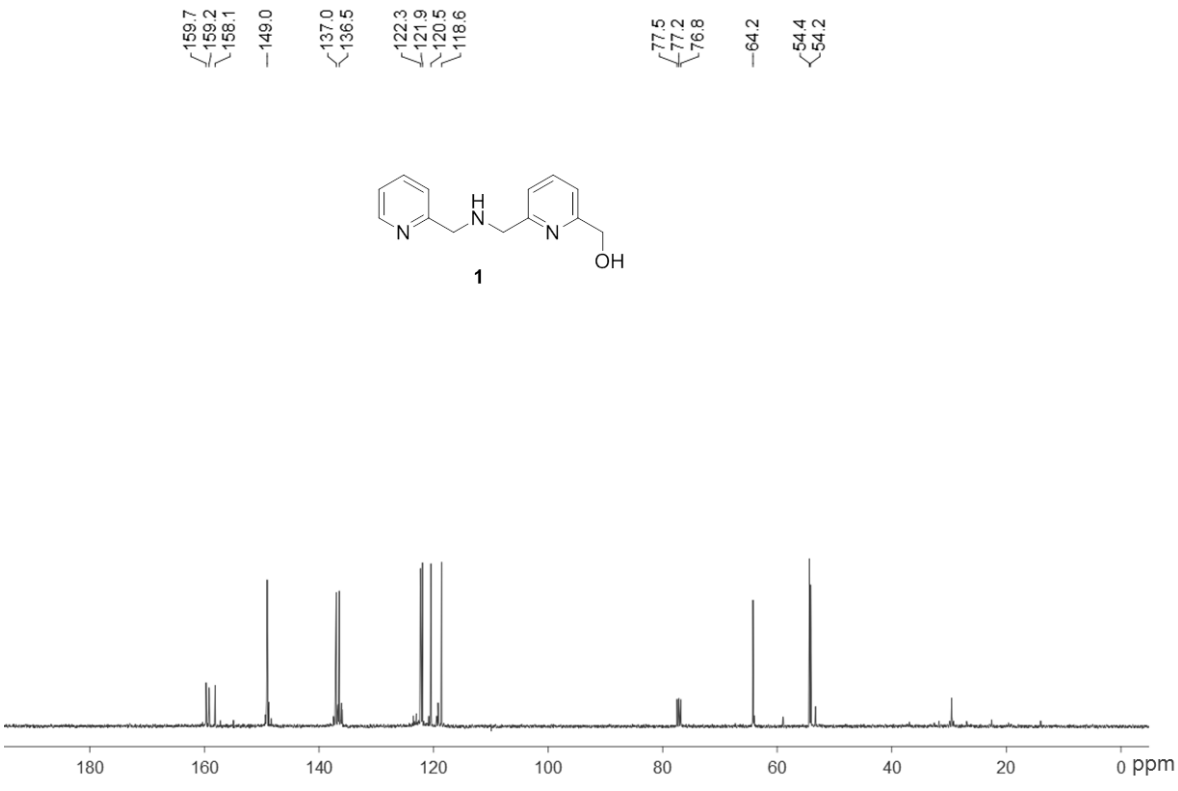


Figure S19. $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298K) of **1**.

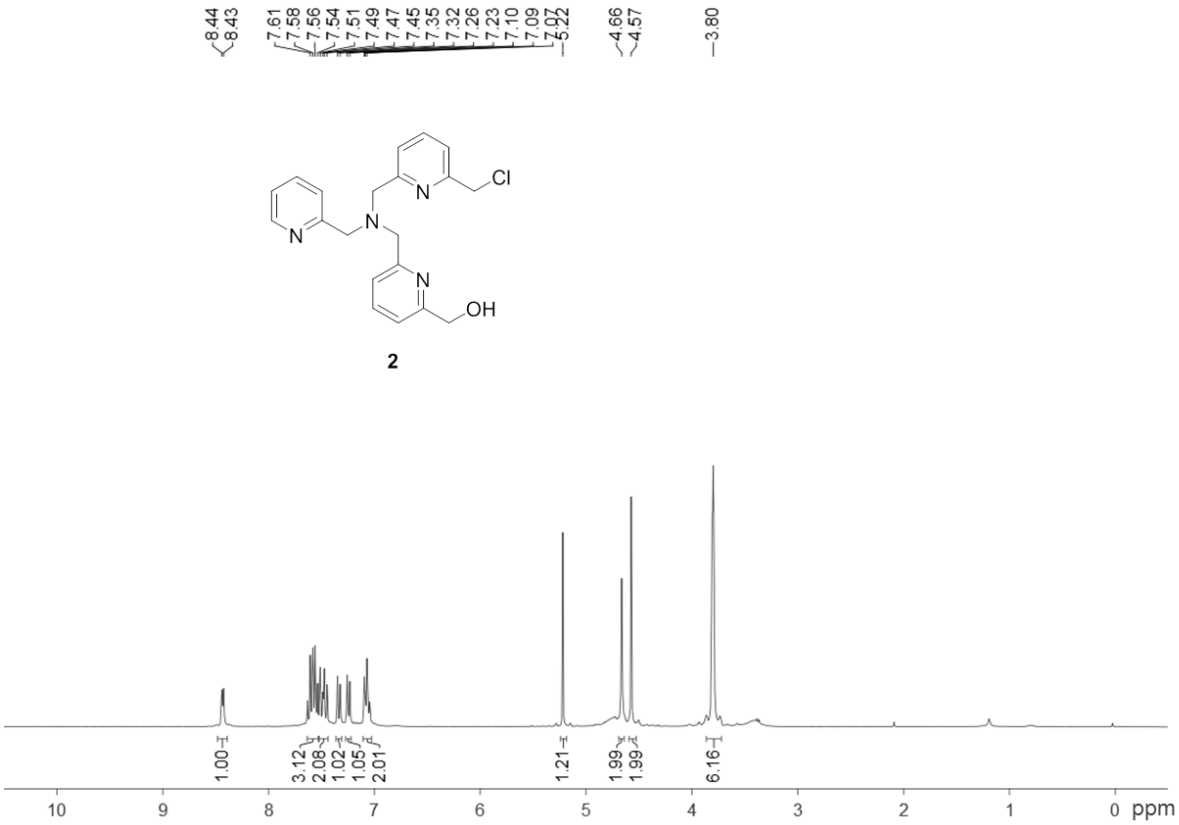


Figure S20. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) of **2**.

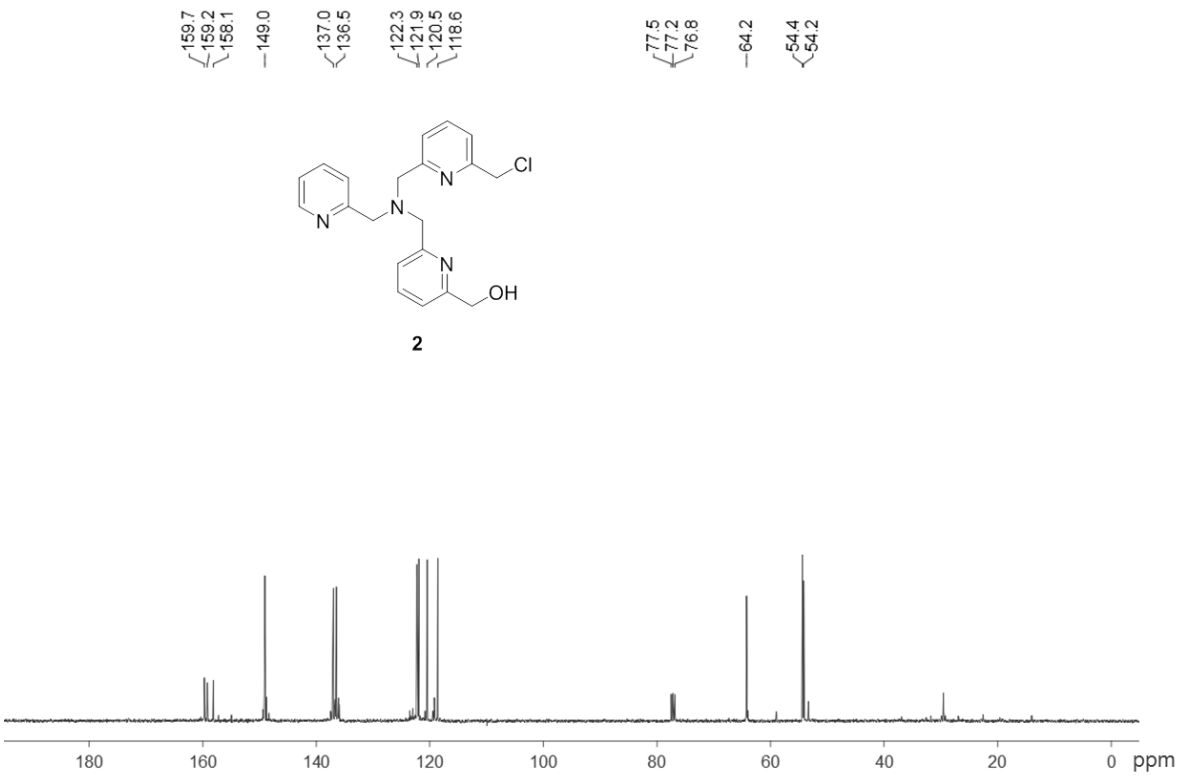


Figure S21. $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298K) of **2**.

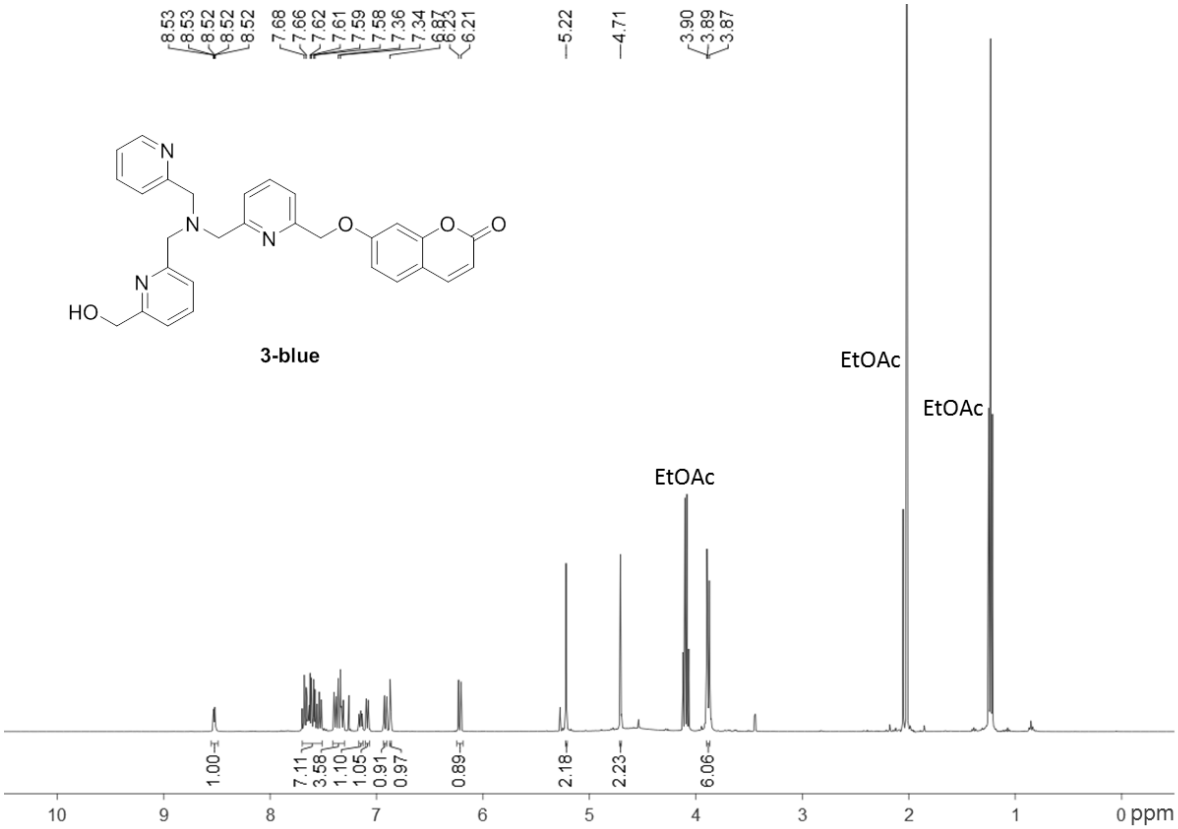


Figure S22. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) of **3-blue**.

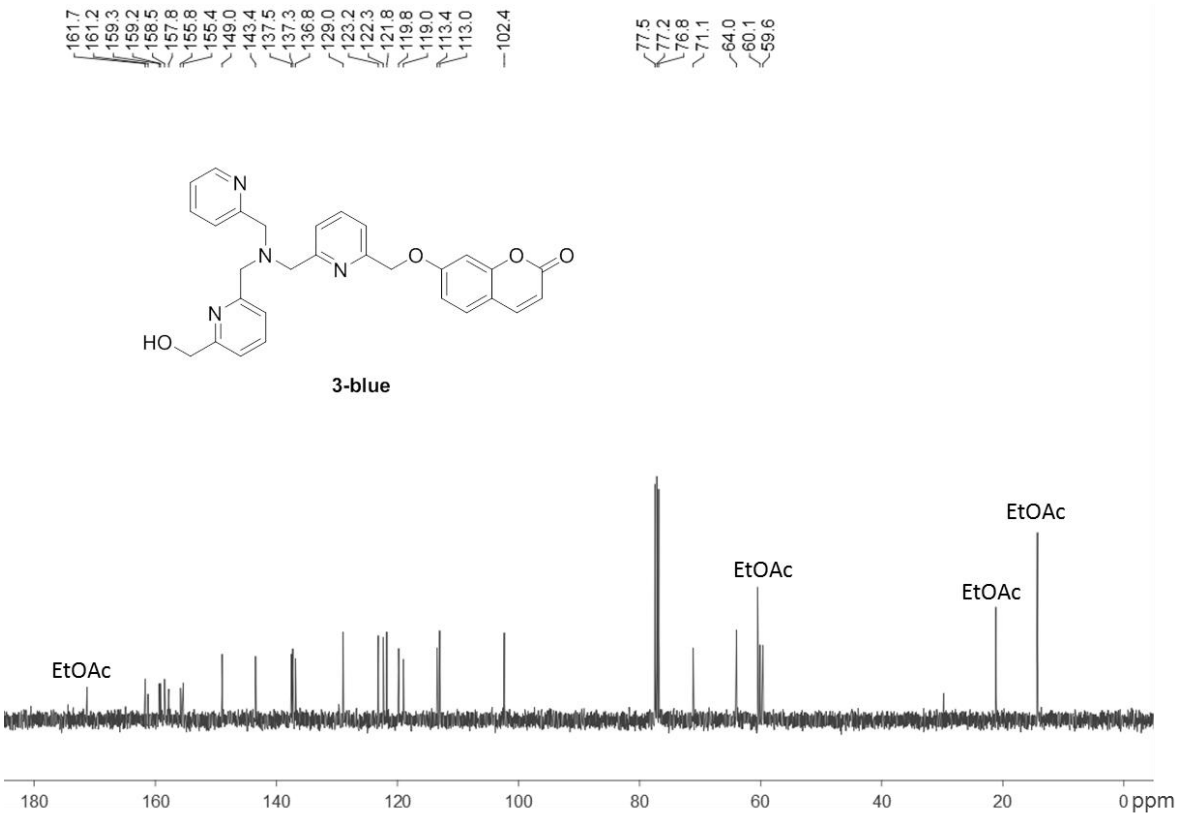


Figure S23. $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298K) of **3-blue**.

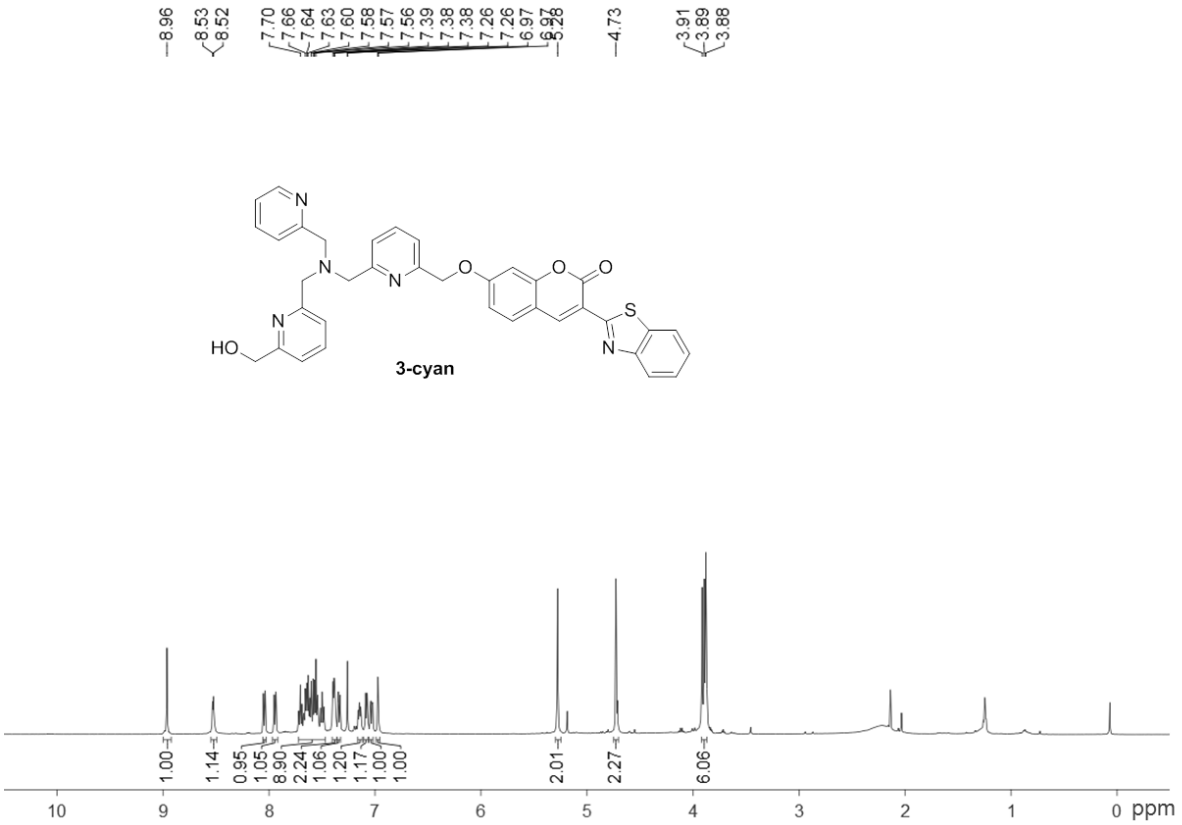


Figure S24. ^{1}H NMR (500 MHz, $CDCl_3$, 298K) of **3-cyan**.

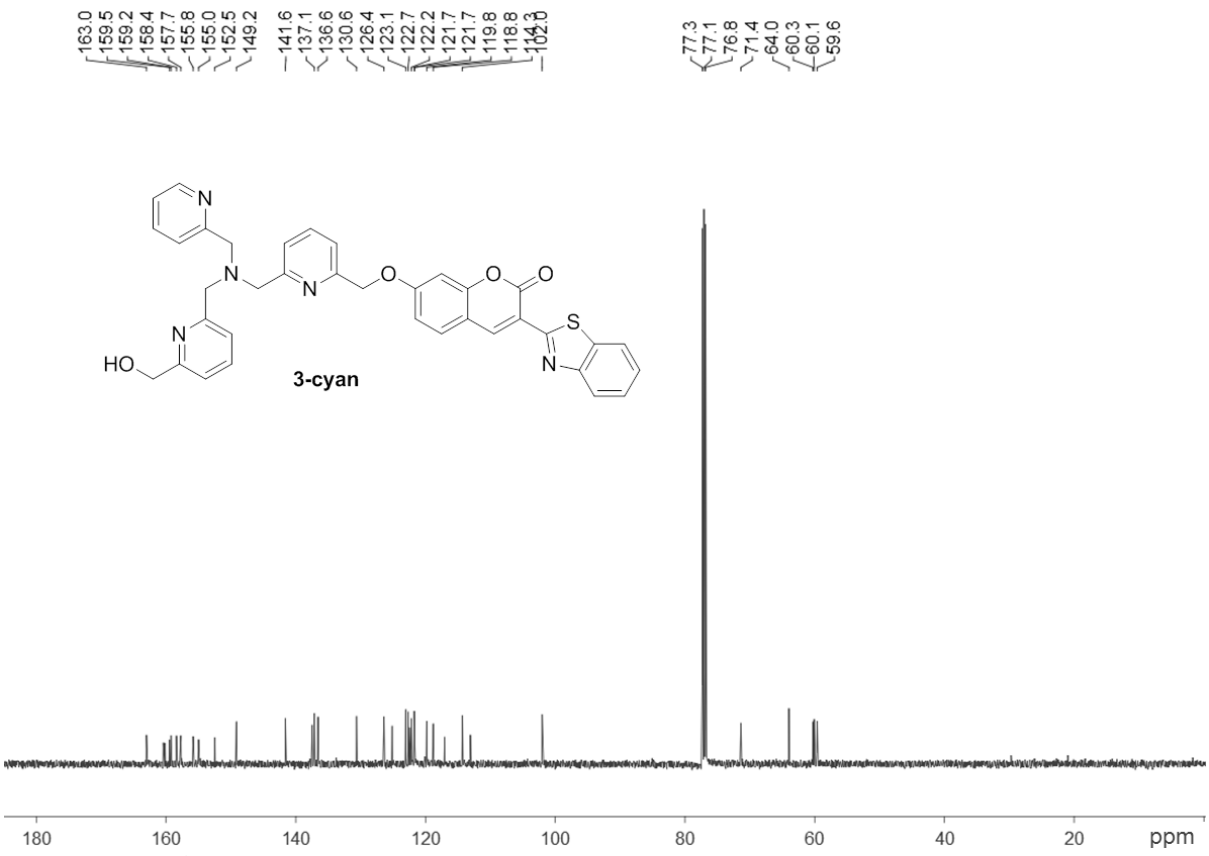


Figure S25. $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$, 298K) of **3-cyan**.

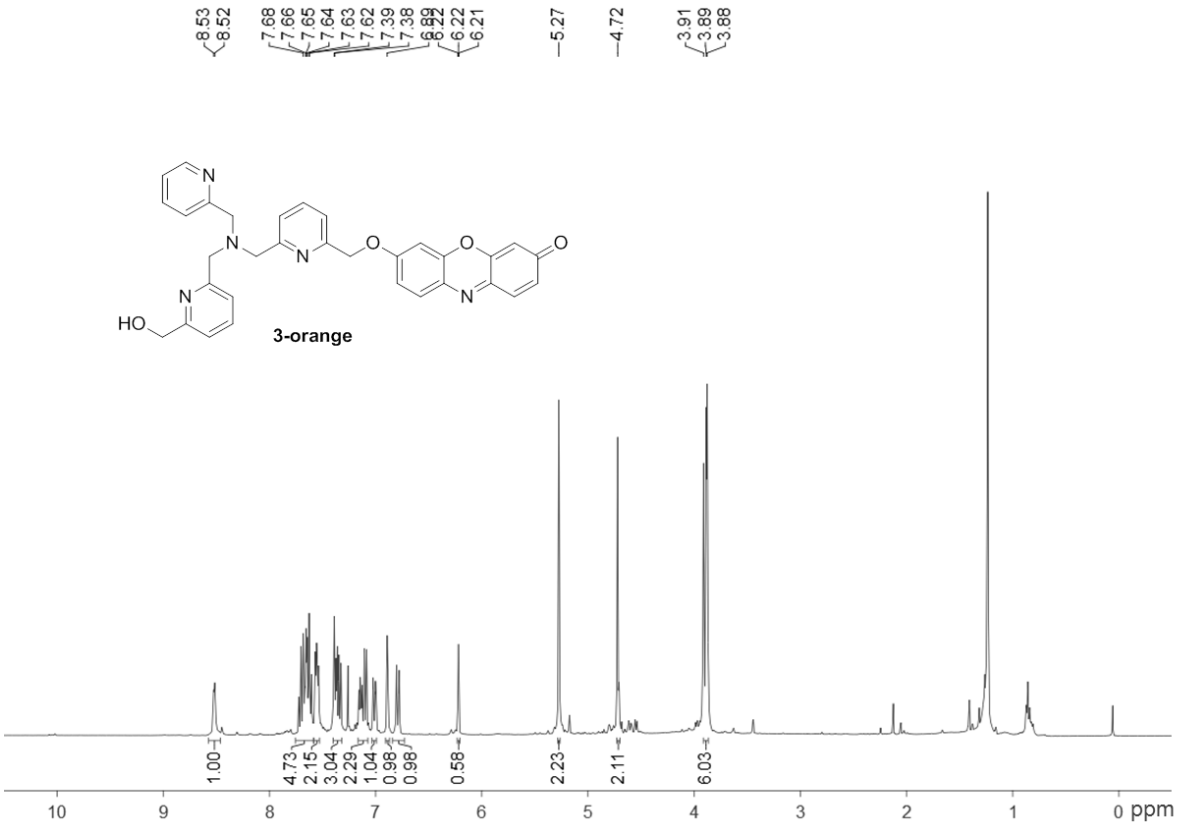


Figure S26. ^{1}H NMR (400 MHz, $CDCl_3$, 298K) of **3-orange**.

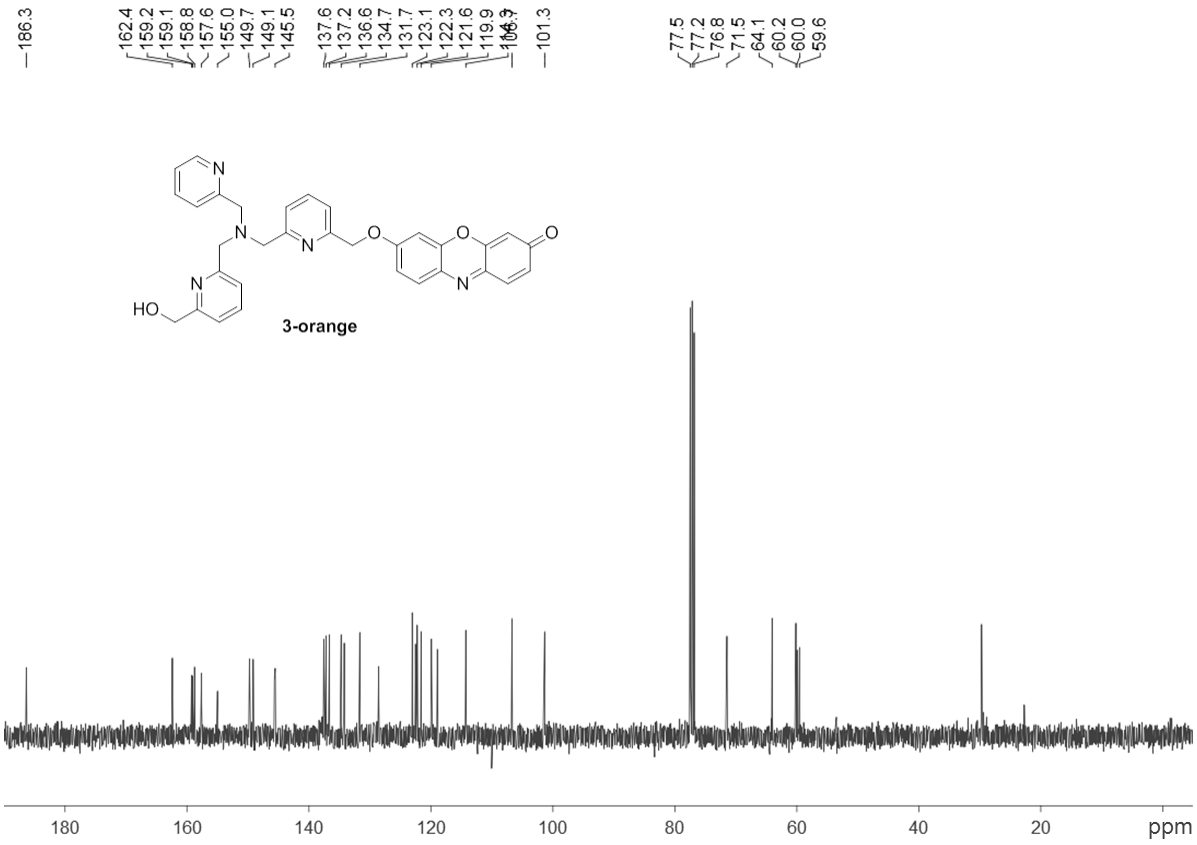


Figure S27. $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298K) of **3-orange**.

7. MS spectra

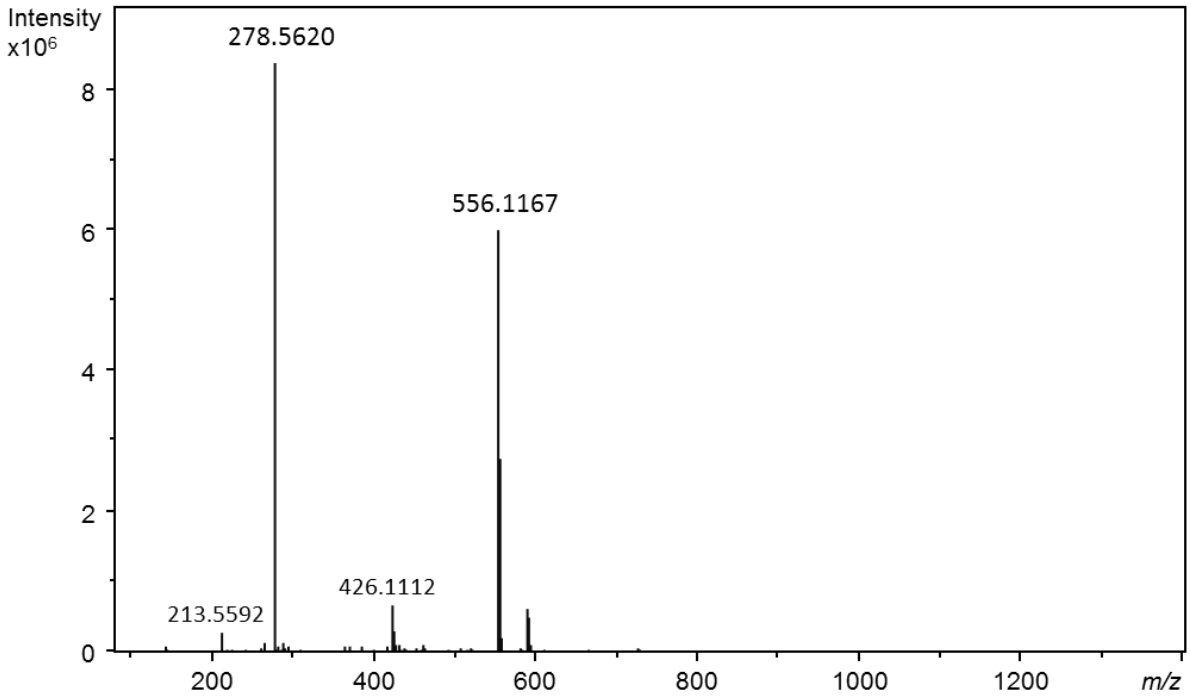


Figure S28. HRMS (+ve) spectrum of **SOP-blue**.

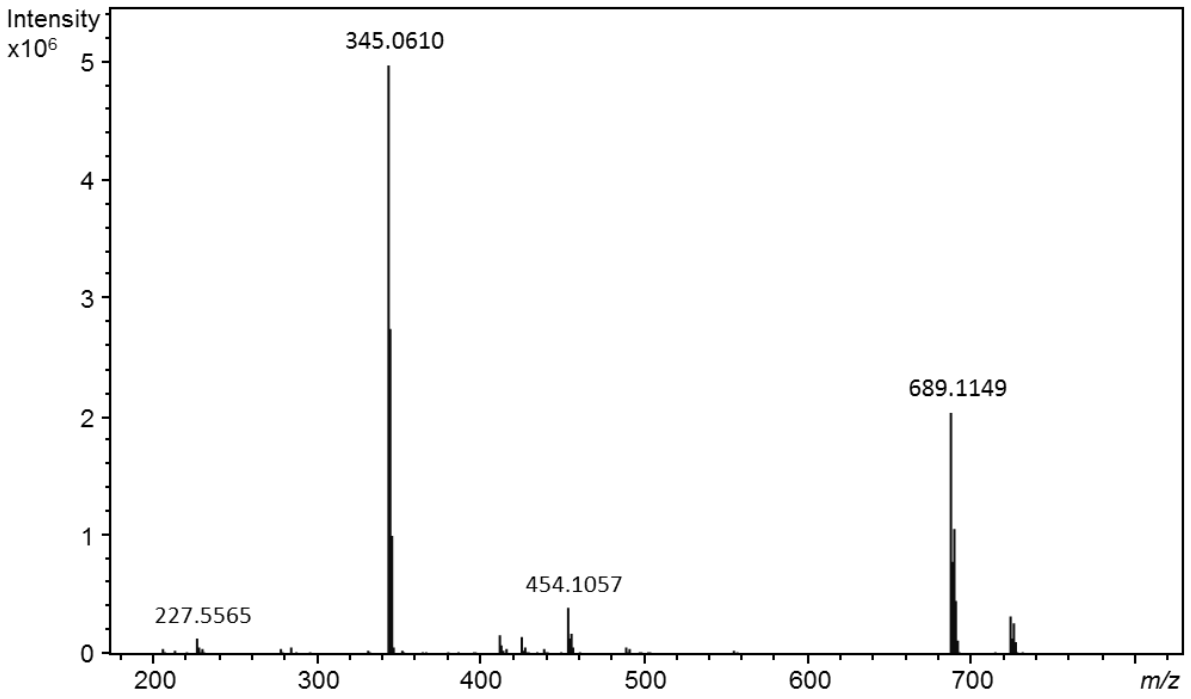


Figure S29. HRMS (+ve) spectrum of **SOP-cyan**.

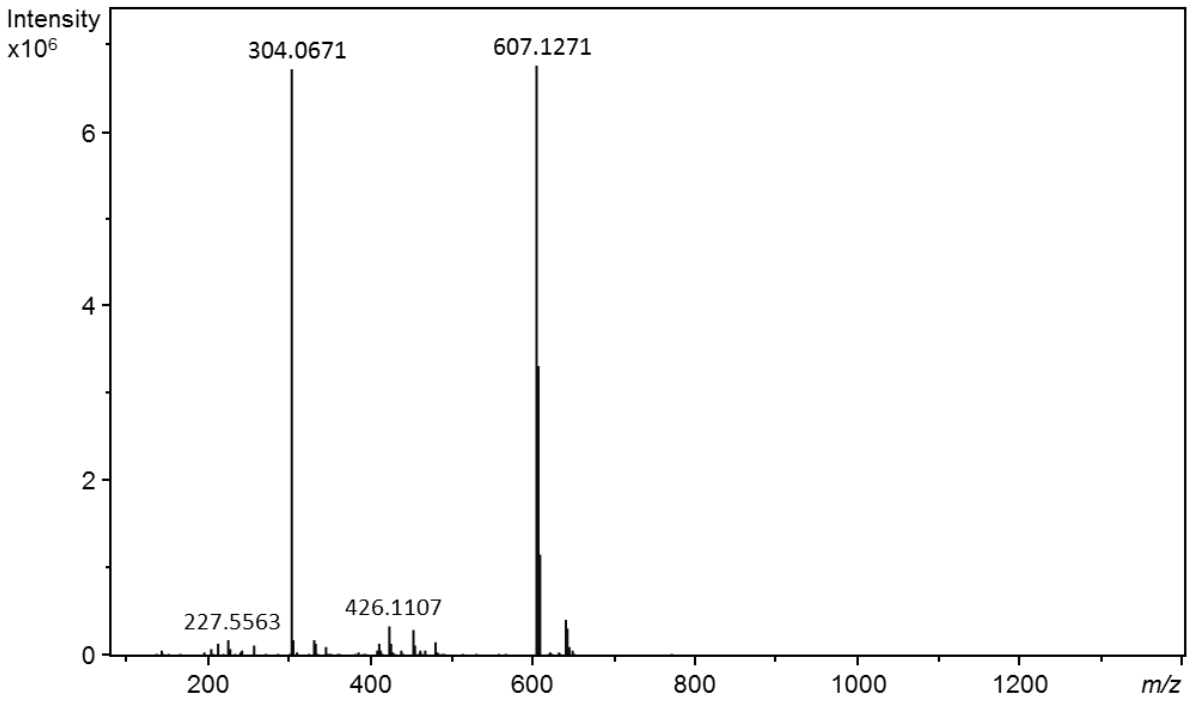


Figure S30. HRMS (+ve) spectrum of **SOP-orange**.

8. References

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