Selective Ratiometric Detection of H_2O_2 in Water and in Living Cells with Boronobenzo[*b*]quinolizinium Derivatives

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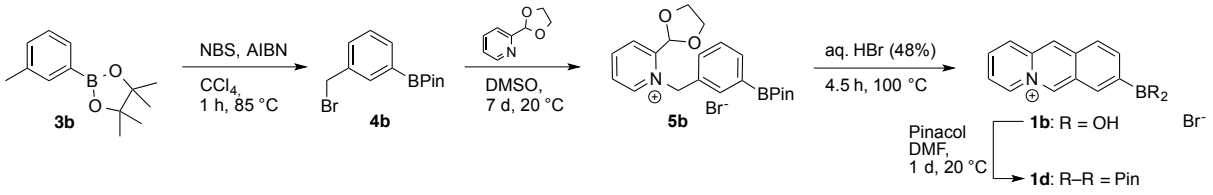
Electronic Supporting Information (ESI)

1. General instrumentations and materials

All commercially available chemicals were obtained from Sigma-Aldrich, Acros or Alfa Aesar, ChemPur (3-tolylboronic acid, 4-tolylboronic acid) were reagent grade and used without further purification. 9-Boronobenzo[*b*]quinolizinium bromide (**1a**),[1] 9-hydroxybenzo[*b*]quinolizinium tetrafluoroborate (**2a**) [2] and 8-hydroxybenzo[*b*]quinolizinium bromide (**2b**)[2] were prepared according to literature procedures. The melting points were determined with a Büchi 510K melting point apparatus and are not corrected. Mass spectra (ESI in the positive-ion mode, source voltage 6 kV) were recorded with a Finnigan LCQ Deca instrument; only *m*/*z* values in the range of 100–2000 units were analyzed. NMR spectra were measured on Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometer at 20 °C; chemical shifts are given in ppm (δ) relative to TMS (δ = 0.00 ppm). Unambiguous proton NMR assignments were established by {1H, 1H}-COSY, HSQC and HMBC experiments. Elemental microanalysis of the new compound was performed with a HEKAtech EuroEA combustion analyzer by Mr. H. Bodenstedt (Organische Chemie I, Universität Siegen). Buffer solution was prepared from purified water and biochemistry-grade chemicals (Fluka BioChemika Ultra). Potassium phosphate buffer (0.1 M): 80.2 ml of K_2HPO_4-solution (*c* = 0.1 M) was combined with 19.8 ml of KH_2PO_4 and adjusted to pH = 7.4 with HCl (2M).

2. Synthesis



Scheme S1. Synthesis of compounds **1b** and **1d**

2-[3-(Bromomethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**4b**).[3] A mixture of 4,4,5,5-tetramethyl-2-(3-methylphenyl)-1,3,2-dioxaborolane (**3b**)[4] (6.60 g, 30.3 mmol), NBS (5.39 g, 30.3 mmol) and AIBN (0.1 g) in CCl_4 (250 ml) was stirred under reflux for 1 h. After cooling to room temperature, the succinimide was removed by filtration and washed with CCl_4. The solvent of the combined organic solutions was removed in vacuo. The product was isolated as yellow solid (7.26 g, 81%) and used for the next reaction without further

purification; m.p. 74–75 °C. – ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.35 (s, 12H, 4 × CH_3), 4.51 (s, 2H, benzyl–CH_2), 7.36 (t, ^3J = 7.6 Hz, 2H, Ar–H), 7.50 (dt, ^3J = 8.2 Hz, ^4J = 1.8 Hz, 1H, Ar–H), 7.74 (d, ^3J = 7,6 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H).

2-(1,3-Dioxolan-2-yl)-1-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]pyridinium bromide (**5b**). Under nitrogen-gas atmosphere a solution of 2-(1,3-dioxolan-2-yl)pyridine[5] (3.70 g, 24.5 mmol) and **4b** (7.26 g, 24.5 mmol) in DMSO (25 ml) was stirred at room temperature for 7 d. The reaction mixture was poured into EtOAc. The white precipitate was collected, washed with EtOAc and diethyl ether, and dried in vacuo to give the product (4.97 g, 45%) as white solid; m.p. 158–159 °C. – ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.33 (s, 12 H, 4 × CH_3), 4.19 (s, 4 H, 2 × OCH_2), 6.00 (s, 2 H, CH_2), 6.33 (s, 1 H, $2_{dioxolan}$-H, 7.44 – 7.45 (m, 2 H, Ar–H), 7.70 (s, 1 H, Ar–H), 7.86 (t, ^3J = 4.6 Hz, 1 H, Ar–H), 7.99 (t, ^3J = 7.0 Hz, 1 H, Ar–H_{py}), 8.65 (d, ^3J = 6.2 Hz, 1 H, Ar–H_{py}).). – ^{13}C-NMR (100 MHz, d_6-DMSO): δ = 25.0 (4×CH_3), 61.4 (–CH_2–), 66.3 (2× OCH_2), 84.4 (dioxaborolan-CH_3), 97.7 ($C2_{dioxolan}$), 126.2 (CH), 128.6 (CH), 129.5 (C_q), 131.1 (C_q), 132.1 (CH), 135.1 (CH), 136.5 (C_q), 146.6 (CH), 152.7 (C_q). – $C_{21}H_{29}BBrNO_2$ × H_2O (465.13 g/mol); calc.: C 54.11, H 6.27, N 3.00; found: C 53.79, H 5.71, N 3.18. – MS (ESI): *m/z* (rel. Int.) = 368 (100) [M^+].

8-Boronobenzo[*b*]quinolizinium bromide (**1b**). A solution of **5b** (3.00 g, 6.71 mmol) in aqueous HBr (48%, 30 ml) was stirred under reflux for 4.5 h. The reaction mixture was cooled to room temperature and poured into THF. The precipitated solid was isolated and recrystallized from water to give **1b** as yellow solid (910 mg, 45%); m.p. 232–233 °C. – ^{1}H-NMR (400 MHz, d_6–DMSO): δ = 7.96 (t, ^3J = 6.9 Hz, 1 H, 3–H), 8.07 (t, ^3J = 8.3 Hz, 1 H, 2–H), 8.33 (d, ^3J = 8.7 Hz, 1 H, 10–H), 8.39 (d, ^3J = 8.7 Hz, 1 H, 9–H), 8.57 (d, ^3J = 8.9 Hz, 1 H, 1–H), 8.71 (br s, 2 H, 2 × OH), 8.84 (s, 1 H, 7–H), 9.21 (s, 1 H, 12–H), 9.35 (d, ^3J = 7.0 Hz, 1 H, 4–H), 10.52 (s, 1 H, 6–H). – ^{13}C-NMR (100 MHz, d_6-DMSO): δ = 122.2 (C3), 124.3 (C11), 125.4 (C10), 125.7 (C10a), 126.8 (C1), 131.1 (C2), 134.3 (C4), 135.0 (C7), 135.9 (C6a, C8), 137.8 (C9), 138.5 (C11a), 140.9 (C6). – $C_{13}H_{11}BBrNO_2$ × 2 H_2O (339.03 g/mol); calc.:
C 45.93, H 4.45, N 4.12; found: C 45.73, H 4.43, N 4.10. – MS (ESI): *m/z* (rel. Int.) = 224 (100) [M^+].

General procedure for the reaction of boronobenzo[*b*]quinolizinium with pinacol (GP1).

Under nitrogen-gas atmosphere a solution of benzo[*b*]quinolizinium boronic acid and pinacol in anhydrous DMF was stirred at room temperature for 24 h. The reaction mixture was poured into EtOAc (500 ml). The precipitated yellow solid was collected, washed with cold MeCN and Et_2O and recrystallized from MeCN / DMF (5:1).

9-[3-(4,4,5,5-Tetramethyl-1,3,2-dioxolanborolan-2-yl)]benzo[*b*]quinolizinium (**1c**) was prepared according to **GP1** from **1a** (100 mg, 330 µmol) and pinacol (40.0 mg, 340 µmol) in anhydrous DMF (10 ml). Yield: 120 mg (94%); m.p. 232–233 °C. – ^{1}H-NMR (400 MHz, d_6–DMSO): δ = 1.39 (s, 12 H, 4 × CH_3), 8.01 (t, ^3J = 7.0 Hz, 1 H, 3–H), 8.08-8.13 (m, 2 H, 2–H, 8–H), 8.44 (d, ^3J = 8.6 Hz, 1 H, 7–H), 8.59 (d, ^3J = 8.8 Hz, 1 H, 1–H), 8.75 (s, 1 H, 10–H), 9.33 – 9.36 (s, 2 H, 4–H, 12–H), 10.52 (s, 1 H, 5–H). – ^{13}C-NMR (100 MHz, d_6-DMSO): δ = 24.8 (4 x CH_3), 84.8 (2 x C_q), 122.9 (C3), 125.7 (C11), 126.4 (C10a), 126.6 (C1), 127.0 (C9), 127.2 (C7), 131.4 (C2), 133.6 (C6a), 133.8 (C8), 134.5 (C4), 134.8 (C10), 137.7 (C11a),

140.1 (C6). – $C_{19}H_{21}BBrNO_2 \times 2\ H_2O$ (421.11 g/mol); calc.: C 54.06, H 5.97, N 3.32; found: C 54.10, H 5.55, N 3.44. – MS (ESI): *m/z* (rel. Int.) = 306 (100) [M^+].

8-[3-(4,4,5,5-Tetramethyl-1,3,2-dioxolanborolan-2-yl)]benzo[*b*]quinolizinium (**1d**) was prepared according to **GP1** from **1b** (200 mg, 660 µmol) and pinacol (80.0 mg, 680 µmol) in anhydrous DMF (20 ml). Yield: 160 mg (64%); m.p. 231–232 °C. – ^{1}H-NMR (400 MHz, d_6–DMSO): δ = 1.39 (s, 12 H, 4 × CH_3), 7.98 (t, ^3J = 7.2 Hz, 1 H, 3–H), 8.11 (t, ^3J = 8.4 Hz, 1 H, 2–H), 8.21 (d, ^3J = 8.6 Hz, 1 H, 9–H), 8.37 (d, ^3J = 8.6 Hz, 1 H, 10–H), 8.59 (d, ^3J = 8.9 Hz, 1 H, 1–H), 8.82 (s, 1 H, 12–H), 9.31 (d, ^3J = 7.1 Hz, 1 H, 4–H), 10.59 (s, 1 H, 5–H). – ^{13}C-NMR (100 MHz, d_6-DMSO): δ = 25.0 (4 x CH_3), 84.7 (2 x C_q), 122.5 (C3), 124.6 (C11), 125.3 (C6a, C10a), 126.4 (C10), 126.9 (C4), 131.6 (C2), 134.5 (C1), 136.1 (C8), 136.3 (C7), 137.4 (C9), 138.1 (C11a), 141.3 (C6). – $C_{19}H_{21}BBrNO_2 \times 1/2\ H_2O$ (394.08 g/mol); calc.: C 55.24, H 5.86, N 3.39; found: C 55.26, H 5.68, N 3.41. MS (ESI): *m/z* (rel. Int.) = 306 (100) [M^+].

3. NMR spectra

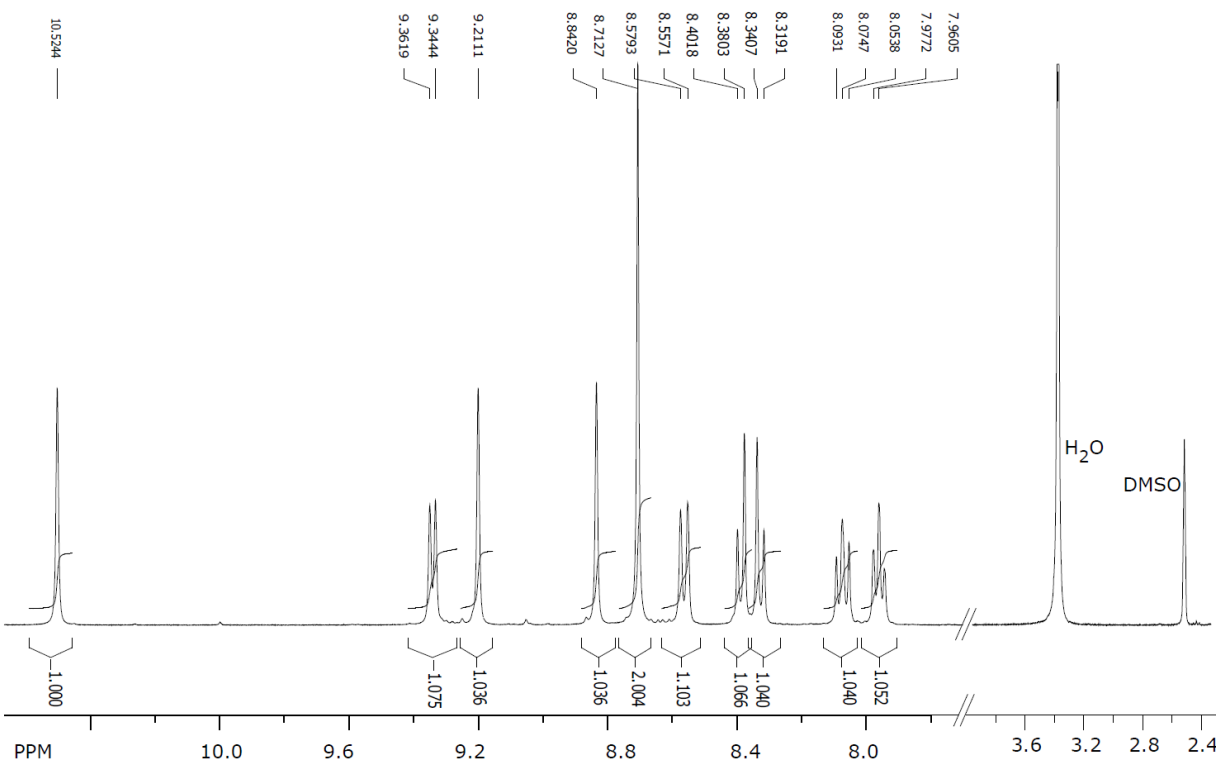


Figure S1. ^{1}H-NMR spectrum (400 MHz) of **1b** in d_6-DMSO.

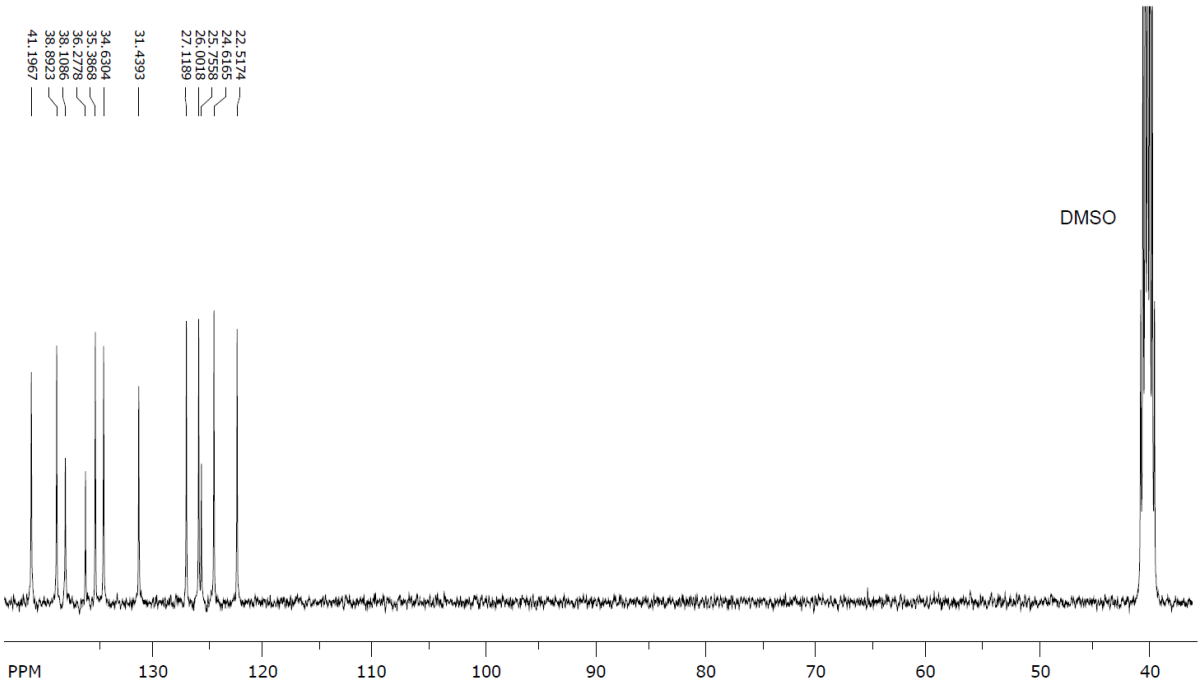


Figure S2. ^{13}C-NMR spectrum (100 MHz) of **1b** in d_6-DMSO.

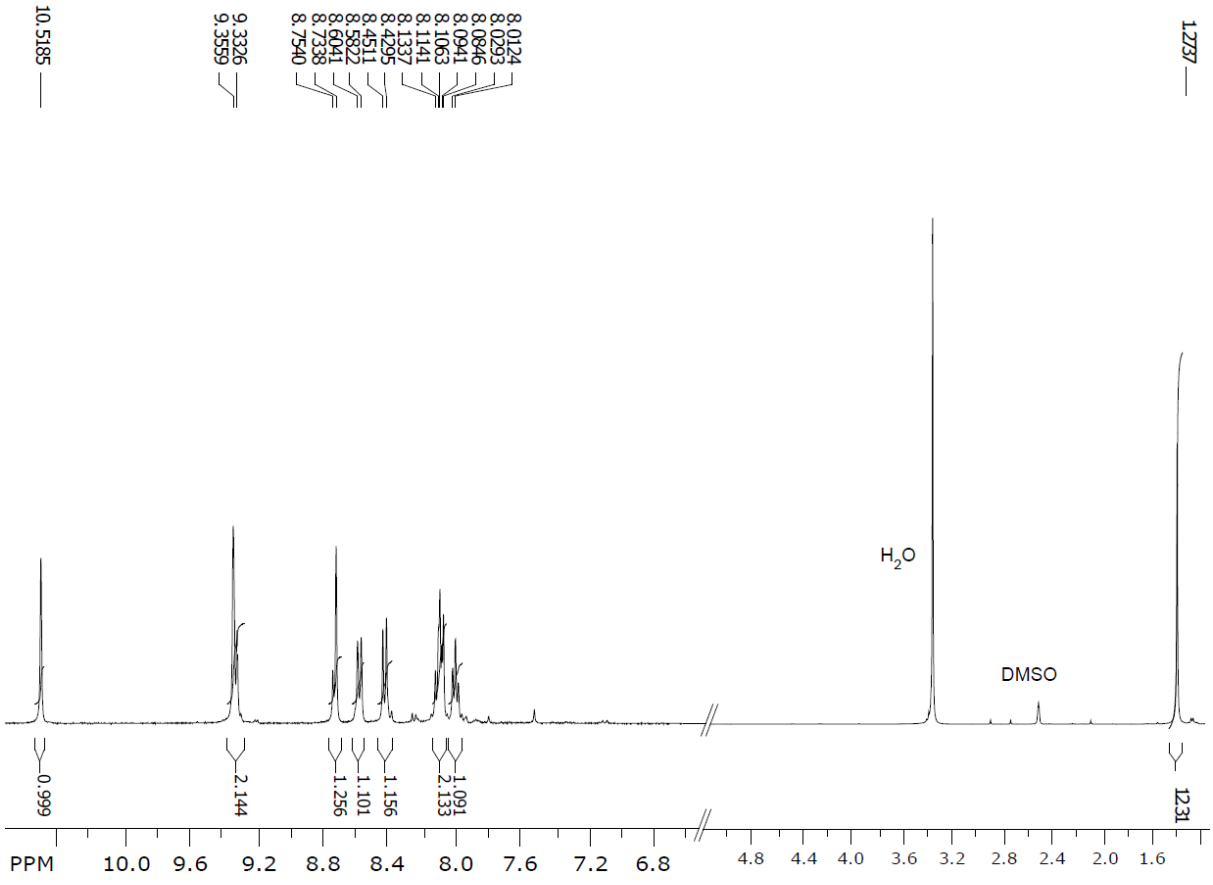


Figure S3. [1]H-NMR spectrum (400 MHz) of **1c** in d_6-DMSO.

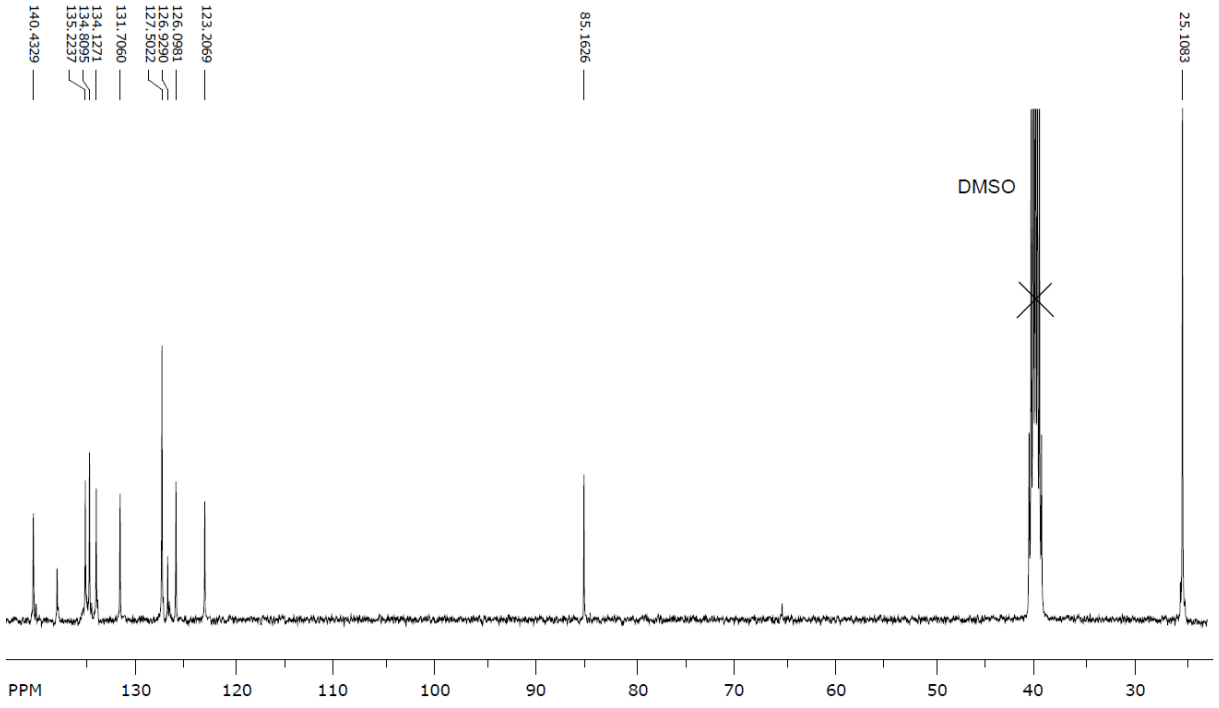


Figure S4. [13]C-NMR spectrum (100 MHz) of **5c** in d_6-DMSO.

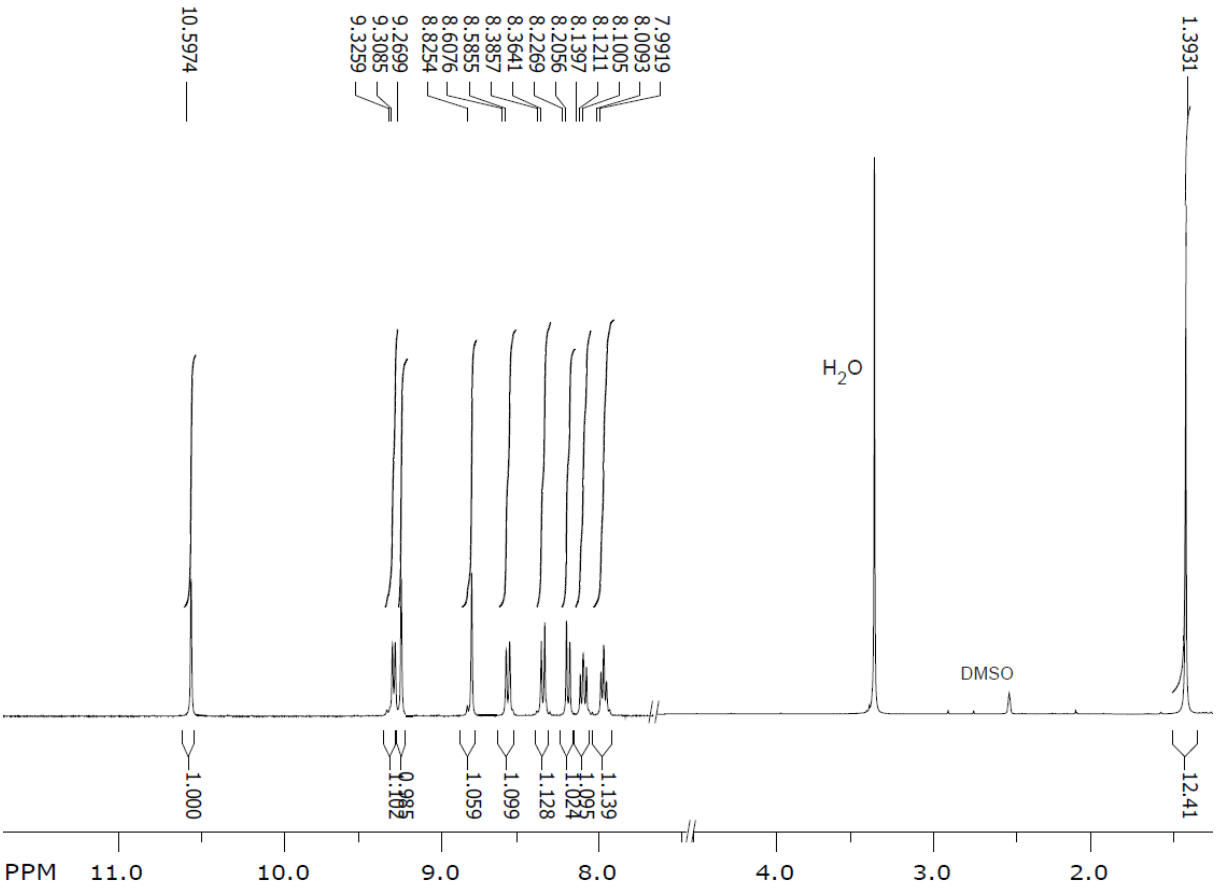


Figure S5. [1]H-NMR spectrum (400 MHz) of **1d** in d_6-DMSO.

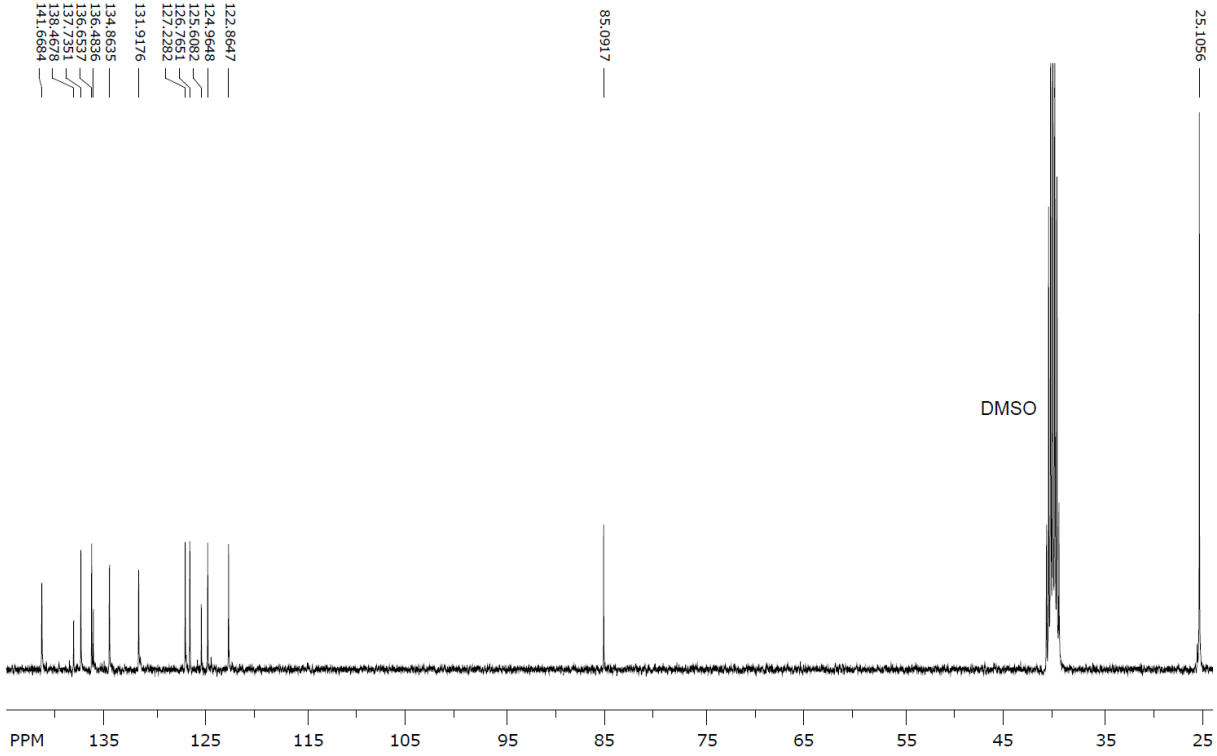


Figure S6. [13]C-NMR spectrum (100 MHz) of **1d** in d_6-DMSO.

4. Reaction of boronobenzo[*b*]quinolizinium derivatives 1a–d with ROS

Generation of ROS[6]

H_2O_2 (30% in water, c = 9.8 M) was diluted with water or phosphate buffer at pH 7.4 to a final concentration of 1.0×10^{-2} M or 1.0×10^{-3} M.

$C_4H_9N_2OO^{\bullet}$ ($c = 2.0 \times 10^{-4}$ M): 2,2'-Azobis(2-amidinopropane)dihydrochloride was dissolved in phosphate buffer (0.1 M, pH 7.4) and stirred at 25 °C for 30 min under aerobic conditions.

$NO^{\bullet}$ ($c = 2.0 \times 10^{-4}$ M): Sodium nitroferricyanide(III) dihydrate was dissolved in phosphate buffer (0.1 M, pH 7.4) and stirred at 25 °C for 30 min.

$HO^{\bullet}$ (c = 1.0 mM): To a solution of $Fe(ClO_4)_2$ (c = 1.0 mM) in phosphate buffer (0.1 M) was added H_2O_2 (c = 0.1 mM), and the reaction mixture was stirred at 25 °C for 30 min.

$^1O_2$ (c = 1.0 mM): To a solution of $(NH_4)_2Fe(SO_4)_2$ (c = 1 mM) in phosphate buffer (0.1 M, pH 7.4) was added TBHP (c = 1.0 mM), and the reaction mixture was stirred at 25 °C for 5 min.

ClO^- (c = 0.1 M): The solution of NaClO (14%, c = 2.1 M) was diluted by water to 1.0 mM at 25 °C.

TBHP (c = 1.0 mM): The solution of TBHP (70%, c = 8.6 M) was diluted by water to 1.0 mM at 25 °C.

$O_2^{\bullet-}$ (c = 1.0 mM): To a solution of xanthine oxidase in phosphate buffer (c = 0.1 M, pH = 7.4) was added a solution of xanthine in phosphate buffer (0.1 M, pH = 7.4) and the mixture was stirred at 25 °C for 30 min.

$ONOO^-$ (c = 4.5 mM): Peroxynitrite was obtained according to literature procedure.[7] The freshly prepared $ONOO^-$ solution was diluted with 0.1 M NaOH, and the concentration was determined by photometric analysis ($\varepsilon_{302} = 1670\ M^{-1}cm^{-1}$).[8]

Reaction with ROS

To a solution of the boronobenzo[*b*]quinolizinium derivative **1a**, **1b**, **1c**, or **1d** in phosphate buffer was added the a solution of the ROS to adjust the final concentration of the quinolizinium derivative (10 μM) and the ROS (1.6×10^{-4} M). The emission spectra of the solution were recorded immediately after preparation of the reaction mixture and after 60 min of incubation at room temp.

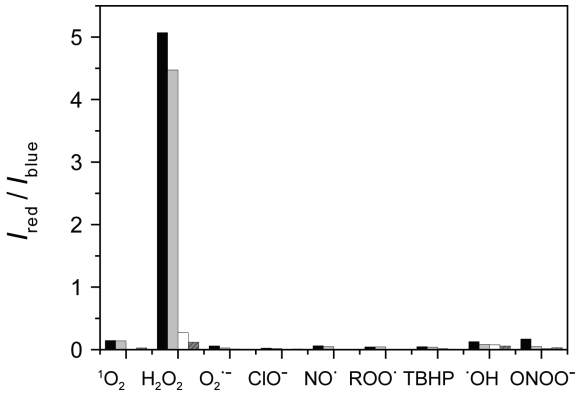


Figure S7. Ratiometric fluorimetric analysis of the reaction of compounds **1a** (black) and **1c** (white) **1b** (grey) and **1d** (hatched grey) (c = 1.0 × 10^{-5} M) with various reactive oxygen species (c = 1.6 × 10^{-4} M, resp.) in phosphate buffer (0.1 M, pH = 7.4), λ_{ex} = 366 nm for **1a** and **1c** or 361 nm for **1b** and **1d**. The bars represent the ratio of emission intensities, I_{red} / I_{blue}, at 60 min after addition of ROS; $ROO^\bullet$ = $C_4H_9N_2OO^\bullet$.

5. Spectroscopic studies

Absorption spectra were obtained with a Varian Cary 100 Bio spectrophotometer. Emission spectra were recorded on a Varian Cary Eclipse. The excitation slit widths, the emission slit widths and the photomultiplier voltage was 5.0 nm, 5.0 nm and 600 V, respectivly. Spectrophotometric measurements were performed in thermostated quartz sample cells at 20 °C. Compounds were dissolved in MeOH to obtain stock solution with concentration of 1.00 × 10^{-3} M. A sample of 50 µl from the stock solution was evaporated and the remaining residue was diluted in aqueous buffer to adjust a concentration of 1.00 × 10^{-5} M. The relative fluorescence quantum yields, Φ_{fl}, were determined according established procedures with a Coumarin 1 as a standard.[9]

The probe molecules show no significant degradation in buffer solution over several days as monitored by absorption spectroscopy; however, it is recommended to use freshly prepared solutions.

Table S1. Spectrophotometric properties of benzo[b]quinolizinium derivatives **1a–d**, **2a–b.**

	λ_{abs} / nm[a]	ε / cm^{-1} M^{-1}[b]	λ_{fl} / nm[c]	Φ_{fl}[d]
1a	401	7.34×10^3	415	0.12
1b	401	6.90×10^3	408	0.06
1c	401	7.70×10^3	415	0.03
1d	400	6.70×10^3	408	0.06
2a	387	1.49×10^4	527	0.14
2b	379	8.40×10^3	600	0.01

[a] Long wavelength absorption maximum, c = 10 μM in phosphate buffer (0.1 M, pH = 7.4). [b] Extinction coefficient, M^{-1} cm^{-1}. [c] Fluorescence maximum, $c = 1.0 \times 10^{-5}$ M, λ_{ex} = 361 nm. [d] Fluorescence quantum yield relative to Coumarin 1 (Φ_{fl} = 0.73 in EtOH[10]).

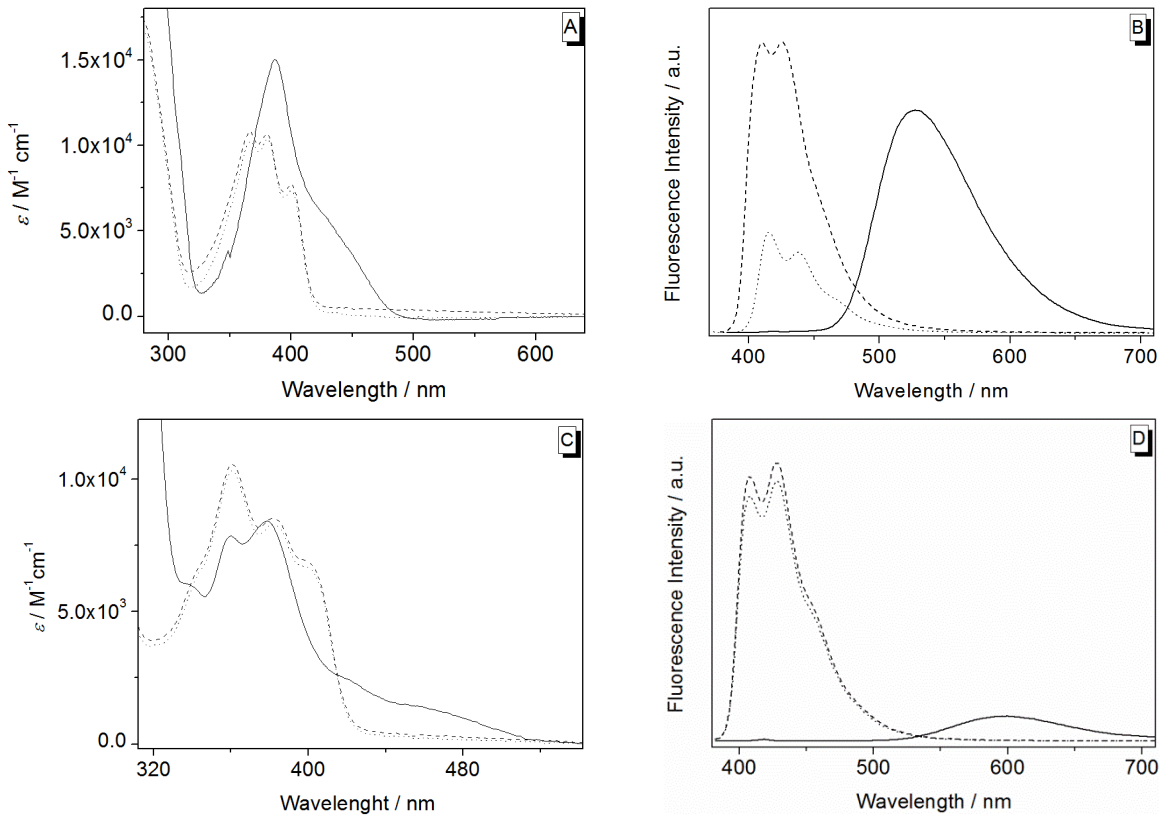


Figure S8. Absorption spectra of **1a** (A, dotted line), **1b** (C, doted line), **1c** (A, dashed line) **1d** (C, dashed line) **2a** (A, continuous line) and **2b** (C, continuous line) (c = 1.0 × 10^{-5} M) in phosphate buffer. Fluorescence spectra of **1a** (B, dotted line), **1b** (D, dotted line), **1c** (B, dashed line) **1d** (D, dashed line) **2a** (B, continuous line) and **2b** (D, continuous line) (c = 1.0 × 10^{-5} M) in phosphate buffer (0.1 M, pH = 7.4); λ_{ex} = 361 nm.

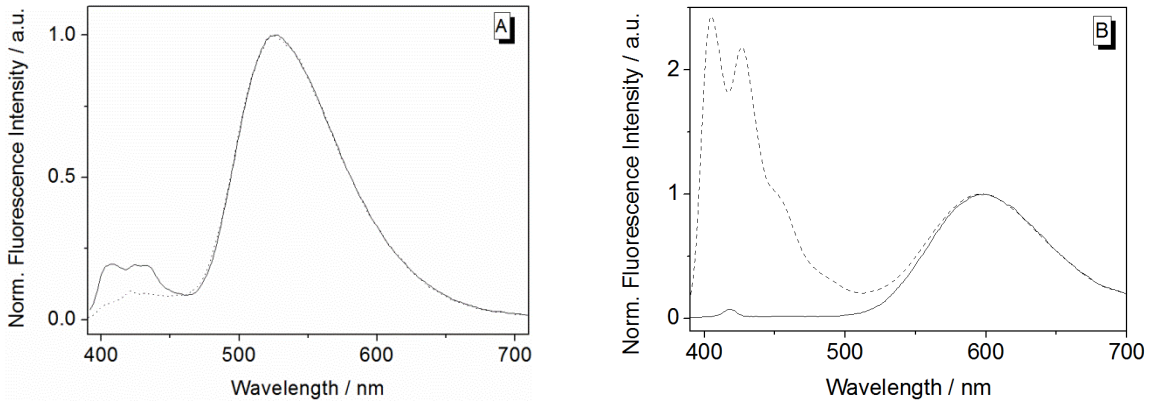


Figure S9. A: Emission spectra of **1c** in phosphate buffer (c = 1.0 × 10^{-5} M, dashed line) at 150 min after addition of H_2O_2 (c = 1.0 × 10^{-2} M) and a solution of **2a** (c = 1.0 × 10^{-5} M, continuous line); λ_{ex} = 361 nm. B: Emission spectra of **1d** in phosphate buffer (c = 1.0 × 10^{-5} M, dashed line) at 30 min after addition of H_2O_2 (c = 1.0 × 10^{-2} M) and a solution of **1b** (c = 1.0 × 10^{-5} M, continuous line) in phosphate buffer; λ_{ex} = 361 nm.

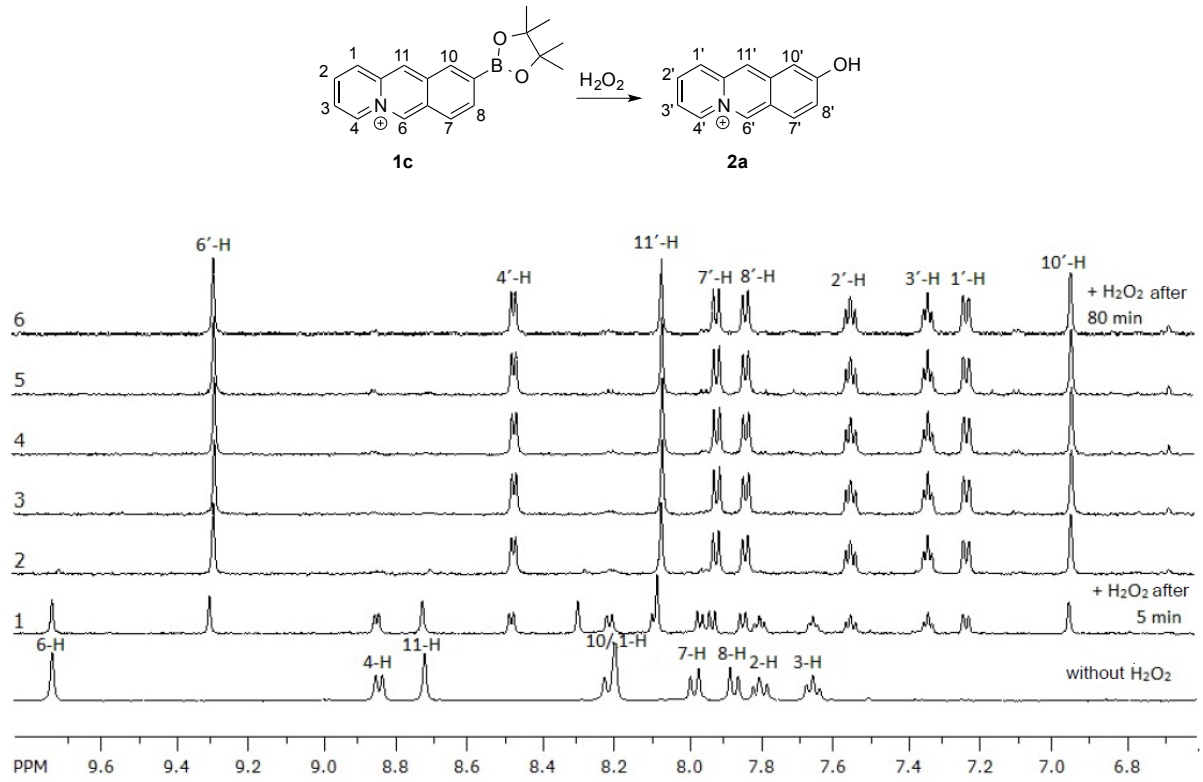


Figure S10. ^{1}H-NMR-spectroscopic monitoring (600 MHz) of the reaction of **1c** (c = 0.01 M) with H_2O_2 (0.01M) in D_2O.

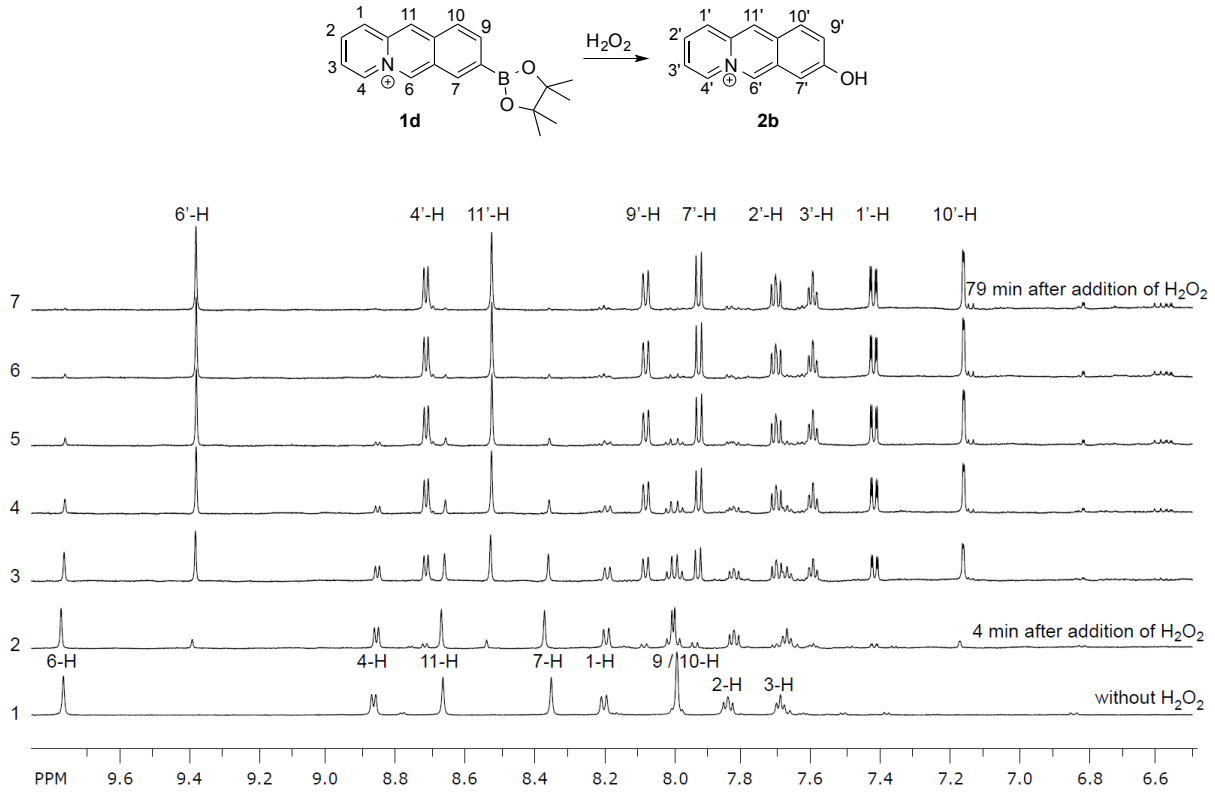


Figure S11. ^{1}H-NMR-spectroscopic monitoring (600 MHz) of the reaction of **1d** (c = 0.01 M) with H_2O_2 (0.01M) in D_2O.

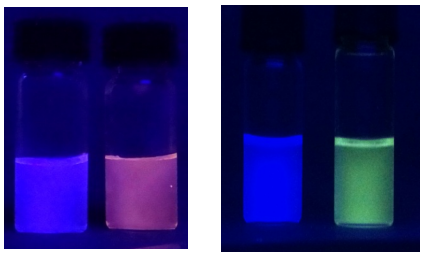


Figure S12. Left: **1b** ($c = 1.0 \times 10^{-4}$ M) in the absence (blue) and presence of H_2O_2 ($c = 1.0 \times 10^{-2}$ M,) (purple) in phosphate buffer (reaction time $t = 2$ h; pH = 7.4; $\lambda_{ex} = 366$ nm). Right: **1a** ($c = 1.0 \times 10^{-4}$ M) in the absence (blue) and presence of H_2O_2 ($c = 1.0 \times 10^{-2}$ M) (green) in phosphate buffer (reaction time $t = 2$ h; pH = 7.4; $\lambda_{ex} = 366$ nm).

6. Kinetic analysis

In the presence of an excess of H_2O_2 the reaction rate was analyzed considering *pseudo* first–order kinetics according to an established protocol.[11] To exclude dilution effects data were used up to 50% conversion. With this method, rate constants of $k_{obs} = 4.3 \times 10^{-4}$ s^{-1} (**1a**) and $k_{obs} = 2.9 \times 10^{-4}$ s^{-1} (**1b**) were obtained from the slope of the logarithmic plot of relative fluorescence intensity at given wavelength, $I_{max} - I_t / I_{max}$, versus reaction time, t (Figure S13; I_{max} = emission intensity of the redshifted maximum of the product at the end of the reaction; I_t = emission intensity of the redshifted maximum after reaction time t).

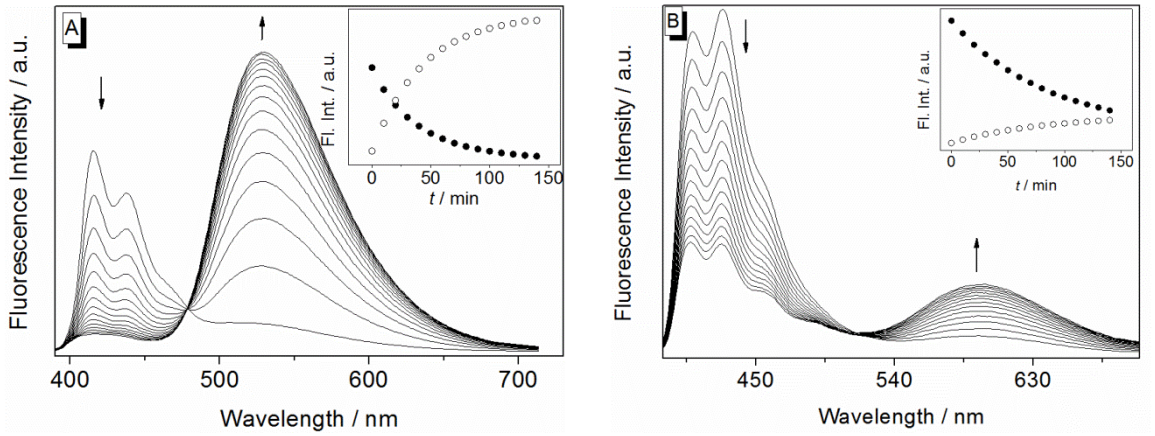


Figure S13. Time course of the fluorescence response ($\lambda_{em} = 415$ nm, 527 nm; $\lambda_{ex} = 361$ nm) of **1a** (10 µM) (A) and ($\lambda_{em} = 408$ nm, 600 nm; $\lambda_{ex} = 361$ nm) of **1b** (B) in 1.0×10^{-3} M H_2O_2 solution in phosphate buffer (0.1 M, pH 7.4). Inset: Plot of fluorescence maxima of **1a** and **1b** at 417 nm (●) and fluorescence maxima of **1a** at 527 nm (○) and **1b** at 600 nm (○) versus time.

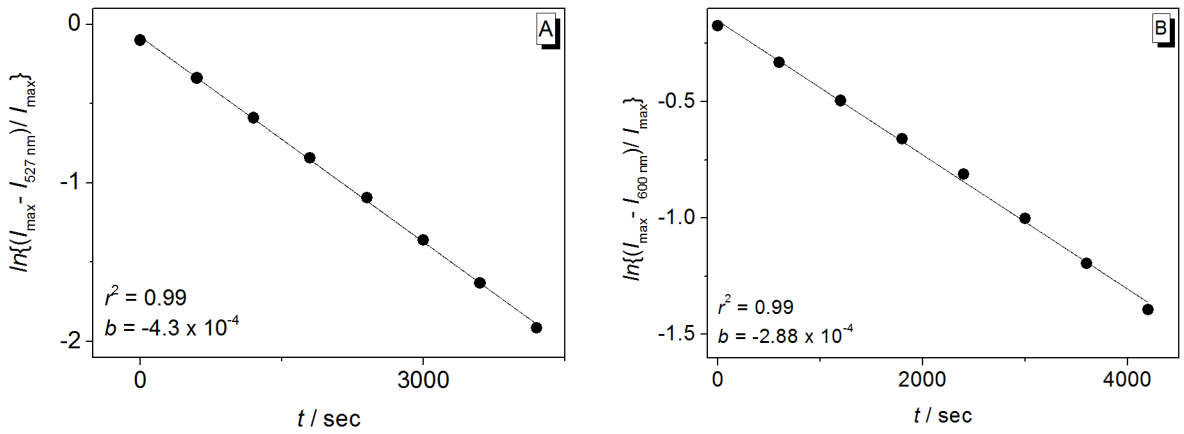
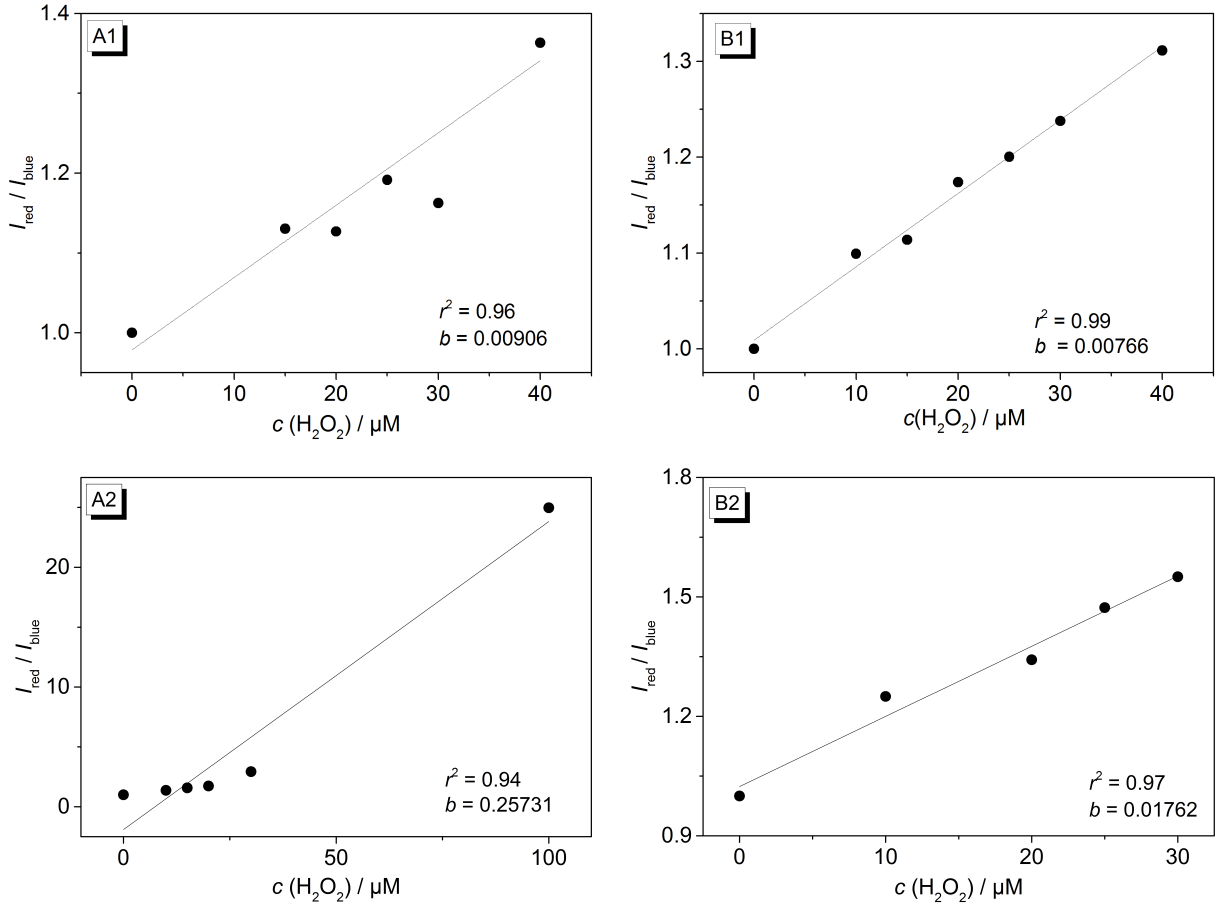


Figure S14. A: Logarithmic plot of relative fluorescence intensity, $I_{max} - I_t$ / I_{max}, of **1a** (A) and **1b** (B) versus reaction time, t (conditions see Fig. S13).

7. Ratiometric analysis



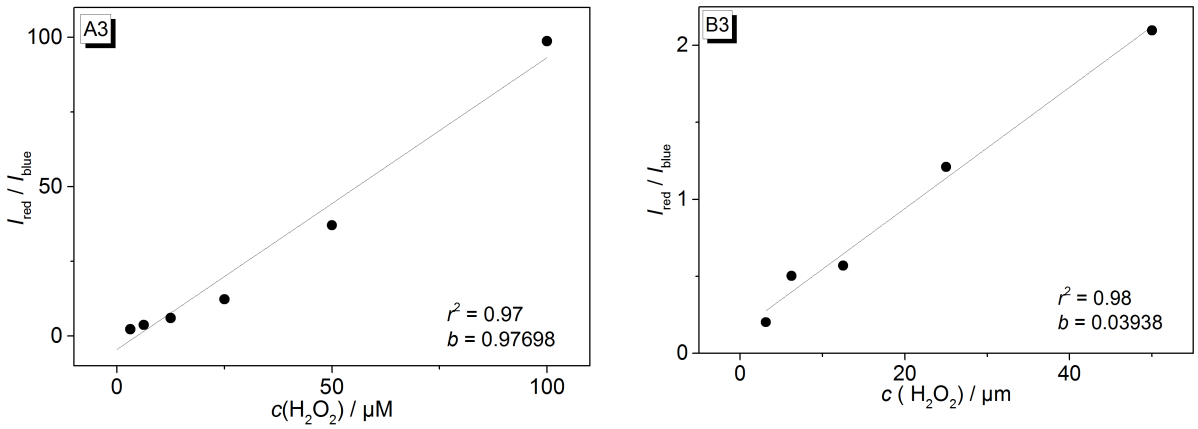


Figure S15. Ratiometric analysis of the fluorimetric response of **1a** (A) and **1c** (B) (c = 50 µM) to H_2O_2 after 5 min (A1, B1), after 10 min (A2, B2) and after 20 min (A3, B3) at 20 °C in phosphate buffer (pH = 7.4).

8. Limit of detection (LOD)

A solution of **1a** or **1c** (c = 50 µM, resp.) and H_2O_2 (varying conc.) in phosphate buffer was stored at 20 °C for 20 min. The reaction mixture was analyzed by emission spectroscopy. The data was analyzed by a plot of the relative emission intensities versus c(H_2O_2). The limit of detection (LOD) was determined according to published procedures (eq. 1). [12]

$$LOD = 3\sigma / b \qquad \text{(eq. 1)}$$

In eq. 1, b is the slope and σ is a standard deviation of the emission intensity of blank samples. The LOD was calculated to be 3.0 µM for **1a** and 5.9 µM for **1c**.

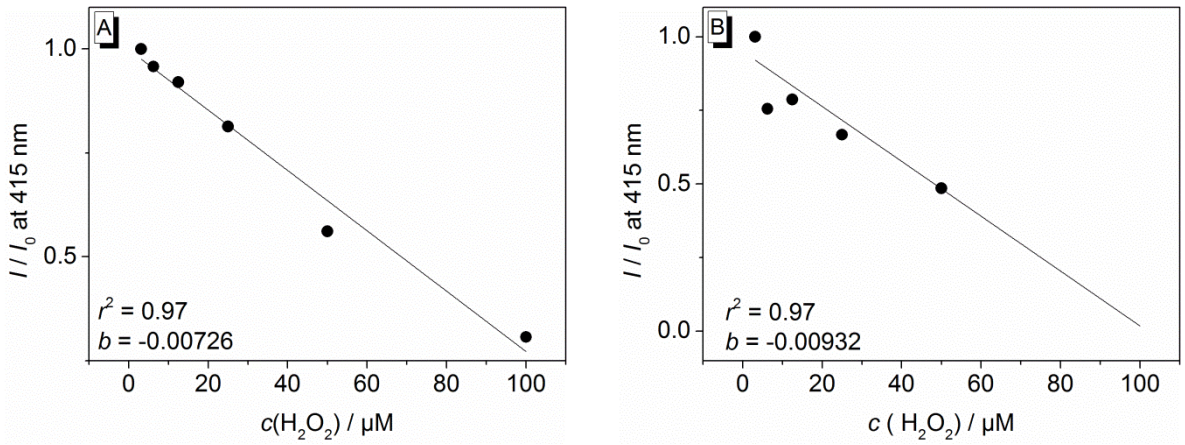


Figure S16. Fluorimetric response of **1a** (A) and **1c** (B) (c = 50 µM) to H_2O_2 after 20 min at 20 °C in phosphate buffer (pH = 7.4) analyzed by a plot of relative emission intensity versus c (H_2O_2).

9. Biological studies

Cell cultures

Human T-leukemia (Jurkat) cells were grown in RPMI-1640 medium (Gibco, Milano, Italy), and human cervix carcinoma (HeLa) cells were grown in DMEM medium (Gibco, Milano, Italy); all supplemented with 115 units ml^{-1} of penicillin G (Gibco, Milano, Italy), 115 μg / mL streptomycin (Invitrogen, Milano, Italy) and 10% fetal bovine serum (Invitrogen, Milano, Italy).

Flow Cytometry Experiments.

Cells were treated and analyzed on a Navios™ flow cytometer (Beckman Coulter) equipped with a 350 nm solid state laser. Fluorescence was detected with 450 nm (FL9) and 550 nm (FL10) band pass filters. For cellular uptake experiments and cytometry experiments Hela cells were trypsinized to detach them from the surface, collected, centrifuged and then analyzed with the flow cytometer. Jurkat cells were centrifuged after incubation of the cells with the test compounds, then washed with Hank's solution and subsequently analyzed. Flow cytometric data were analyzed by FlowJo version 7.6.3 software (TreeStar Inc, San Carlos, CA).

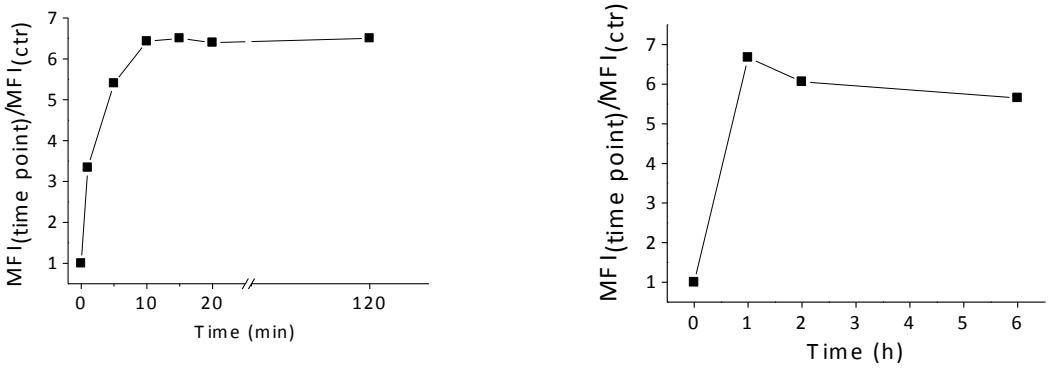


Figure S17. Uptake of **1c** in Jurkat cells (left) and HeLa cells (right). Cells were incubated for the indicated time and analyzed by flow cytometry. The relative emission intensity was expressed as fractional increase of the Median Fluorescence Intensity (MFI) of the treated cells *versus* untreated cells at 450 nm as measured in the FL9 channel at 450 nm.

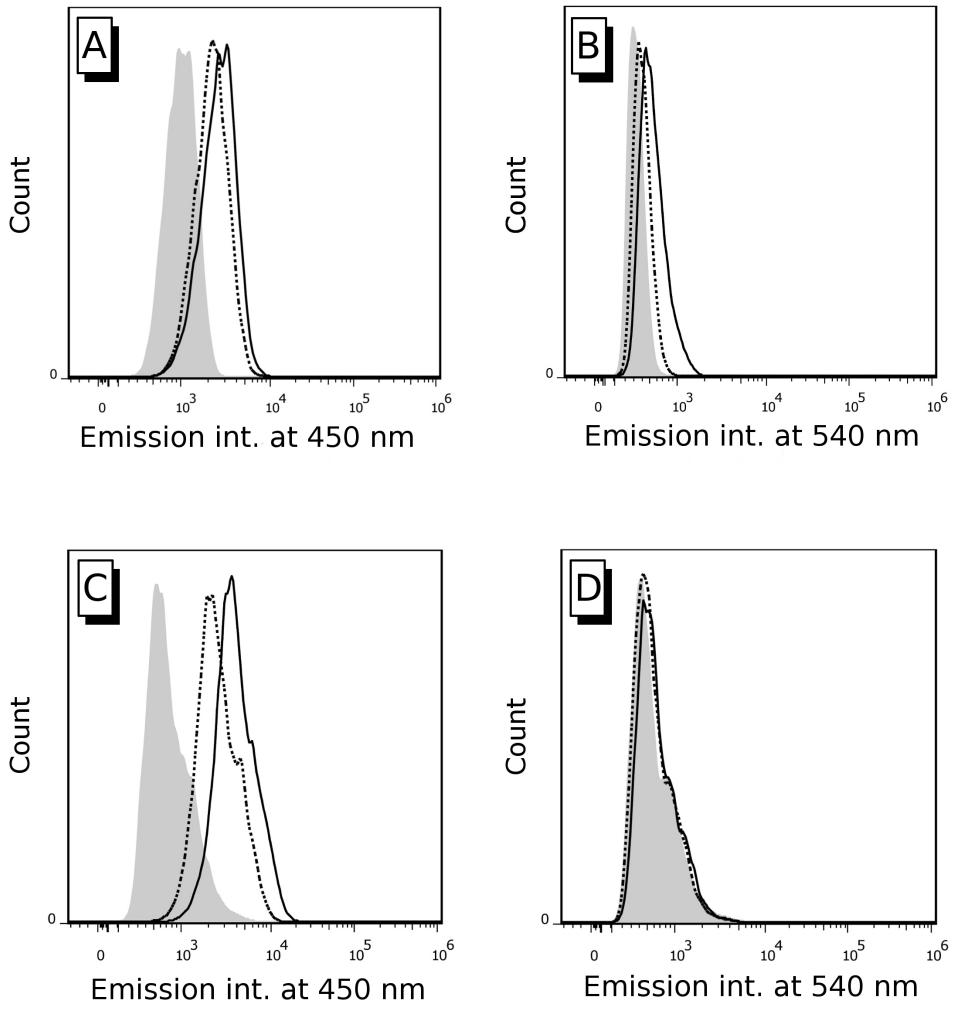


Figure S18. Flow cytometry analysis of Hela (A and B) and Jurkat cells (C and D) incubated with **1c** (continuos line) and **1d** at (dotted line) at c = 10 μM for 1 h. The gray shadows represents untreated cells. Representative flow cytometry trace of three independent experiments with similar results. The data represent at least 10.000 cells for each analysis.

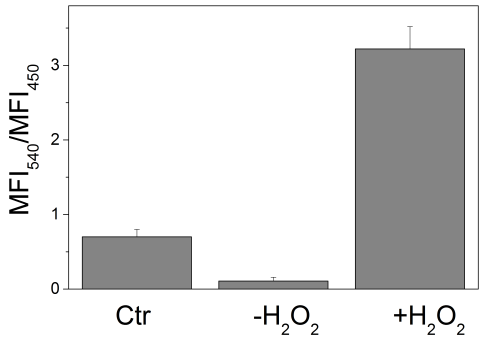


Figure S19. Ratiometric analysis of flow cytometric results with HeLa cells that were incubated with **1c** for 1 h ($-H_2O_2$) and subsequently treated with 100 μM H_2O_2 for 10 min ($+H_2O_2$) (Ctr: Control sample; raw data, see Fig. 3A). The bars represent the ratio of the Median Fluorescence Intensity (MFI) analyzed at 540 nm and 450 nm. Data represented of mean ± SE of three independent experiments.

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[12] (*a*) Z. J. Chen, W. Ren, Q. E. Wright and H. W. Ai, *J. Am. Chem. Soc.* 2013, **135**, 14940; (*b*) N. Karton-Lifshin, E. Segal, L. Omer, M. Portnoy, R. Satchi-Fainaro and D. Shabat *J. Am. Chem. Soc.* 2011, **133**, 10960; (*c)* K. B. Daniel, A. Agrawal, M. Manchester and S. M. Cohen *ChemBioChem* 2013, **14**, 593.