SUPPORTING INFORMATION

A new fluorescent "turn-on" chemodosimeter for the detection of hydrogen sulfide in water and in living cells

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Reagents

The chemicals 8-hydroxyquinoline and 1-fluoro-2,4-dinitrobenzene were purchased from Sigma-Aldrich. Triethylamine (98%) was purchased from J.T.Baker. Analytical-grade solvents, sodium hydroxide and hydrochloric acid (37%) were purchased from Scharlau (Barcelona, Spain). For cell culture experiments, DMEM with L-glutamine, piruvate and Fetal Bovine Serum (FBS) trypan blue solution (0.4%) cell culture grade and trypsin were purchased from Roche Applied Science. DMSO for cell culture and PBS were purchased from Sigma-Aldrich. The cell proliferation reagent WST-1 was purchased from Roche Applied Science.

Methods

UV-visible spectra were recorded with a Jasco V-650 Spectrophotometer. Fluorescence measurements were carried out in a Jasco FP-8500 Spectrophotometer. ^{1}H and ^{13}C-NMR spectra were acquired in a Bruker Advance III (400 MHZ). Mass spectra were obtained from a Tripletof T5600 (ABSciex, USA) spectrometer. Cell viability measurements were carried out with a Wallac 1420 Workstation.

Synthesis of 8-(2,4-dinitrohydroxy)quinoline (1)

8-Hydroxyquinoline (0.725 g, 5 mmol) and triethylamine (0.5 ml, 3.6 mmol) were dissolved in acetone (5 mL). To this solution, at room temperature, 1-fluoro-2,4-dinitrobenzene (0.930 g, 5 mmol) dissolved in acetone (5 mL) was added. The reaction mixture was refluxed for 30 minutes. After evaporation of acetone, 5% HCl (10 mL) was added and the precipitate was filtered, washed with water then suspended in 5% NaOH (15 mL) and stirred for 15 minutes. The solid was filtered and washed with water and finally purified by silica gel chromatography using hexane-acetone 1:1 v/v as eluent. The final product was isolated as a yellow solid (1.3 g, 4.4 mmol, 85% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.93 (d, J = 2.8 Hz, 1H), 8.79 (dd, J = 4.2, 1.7 Hz, 1H), 8.25 (dd, J = 8.4, 1.7 Hz, 1H), 8.18 (dd, J = 9.3, 2.8 Hz, 1H), 7.83 (dd, J = 7.9, 1.7 Hz, 1H), 7.65 – 7.56 (m, 2H), 7.47 (dd, J = 8.4, 4.2 Hz, 1H), 6.79 (d, J = 9.3 Hz, 1H).
^{13}C NMR (101 MHz, $CDCl_3$) δ: 157.45, 151.00, 149.39, 141.36, 140.56, 138.88, 136.37, 130.31, 128.66, 126.81, 122.40, 120.94, 119.37.
HRMS-EI *m/z*: calcd for $C_{15}H_9N_3O_5$ 311.0542; found: 312.0615 ($M+H^+$).

Characterization of chemodosimeter 1

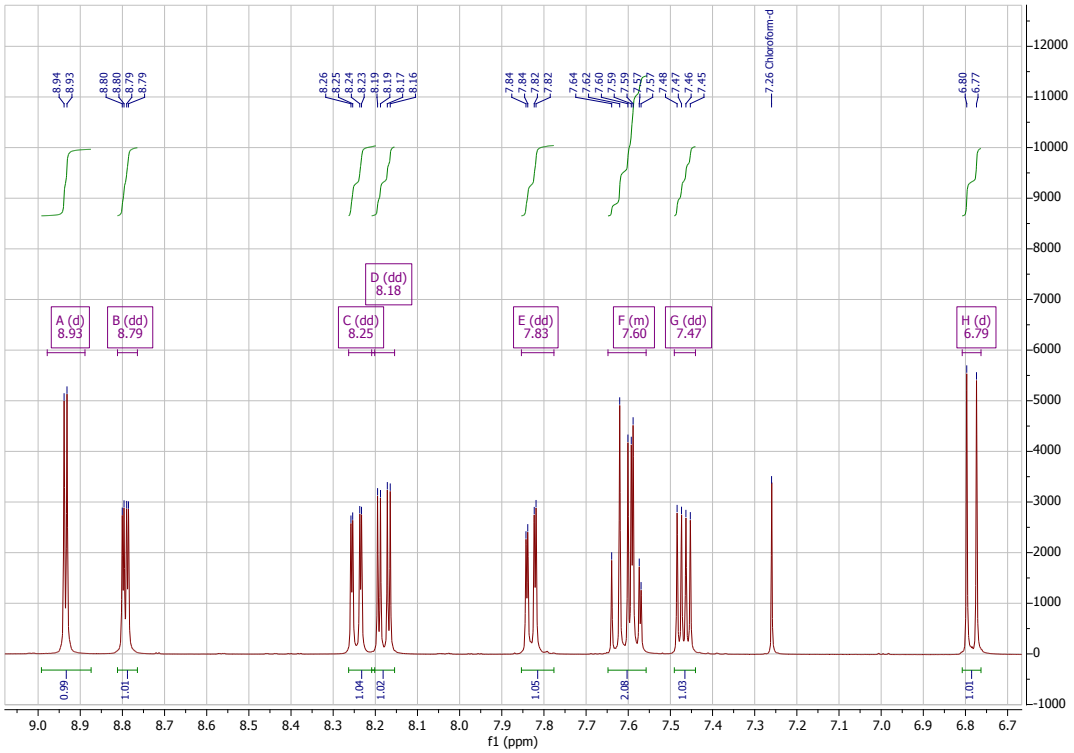

Figure SI-1. ^{1}H-NMR spectrum of probe **1** in $CDCl_3$.

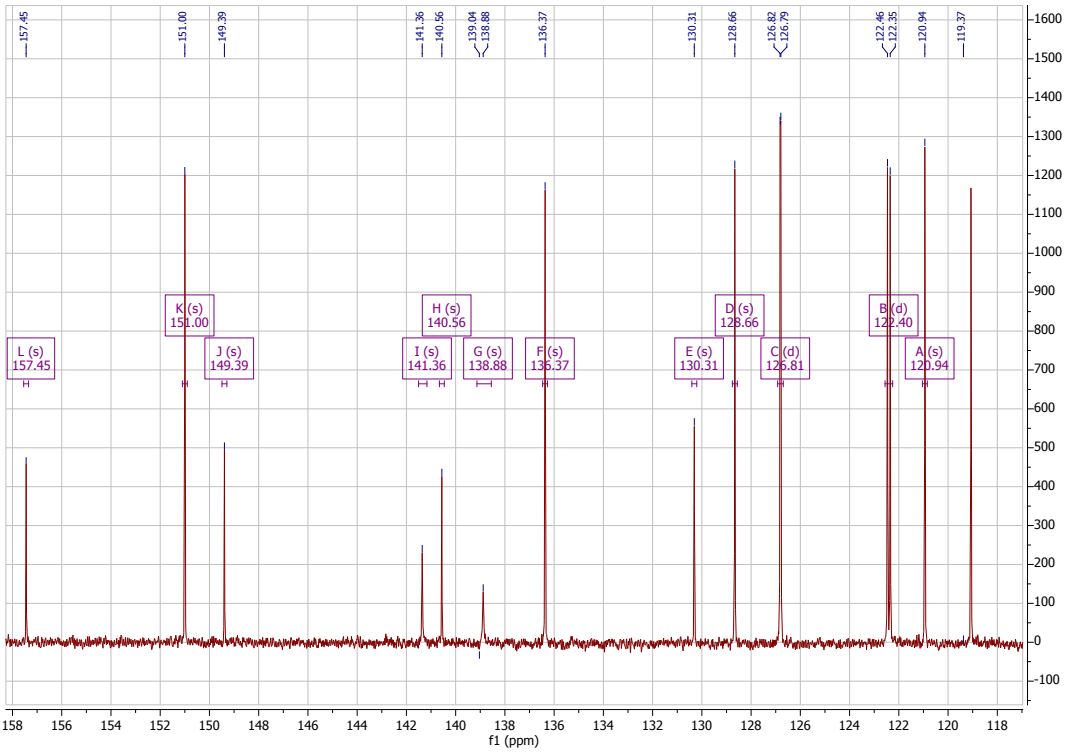

Figure SI-2. ^{13}C-NMR spectrum of probe **1** in $CDCl_3$.

Fluorescence measurements of 1 in the presence of HS^-

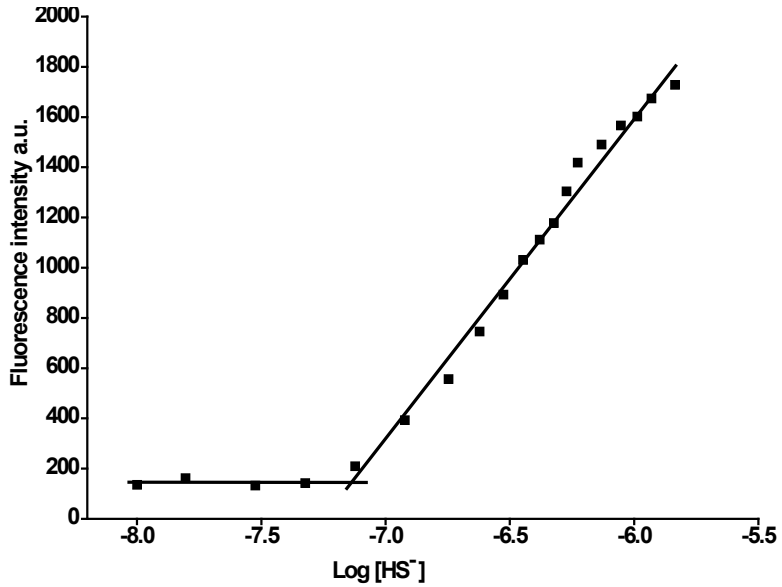

Figure SI-3. Emission intensity at 514 nm (excitation at 450 nm) of HEPES (7 mM, pH 7.4)-DMSO 99:1 v/v solutions of probe **1** (5.0 μM) upon addition of increasing quantities of HS^- anion after 50 min of the reaction.

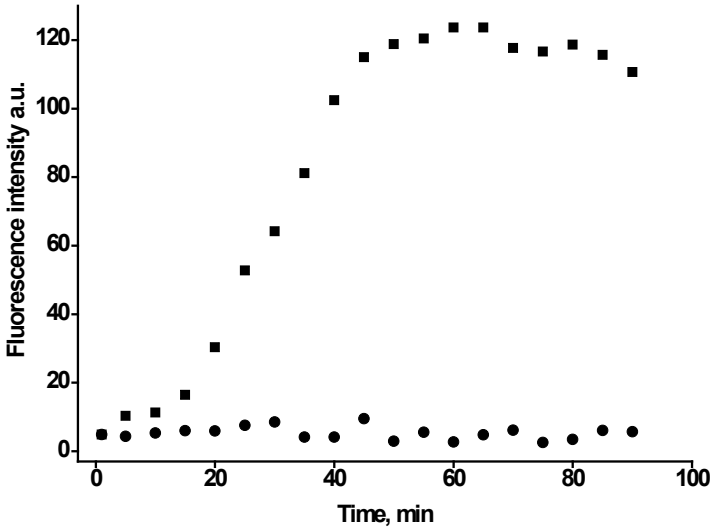

Figure SI-4. Time-dependent emission intensity at 514 nm (upon excitation at 450 nm) of HEPES (7 mM, pH 7.4)-DMSO 99:1 v/v solutions of chemodosimeter **1** (5.0 μM) in absence (●) and in the presence of 10 equivalents of HS^- anion (■).

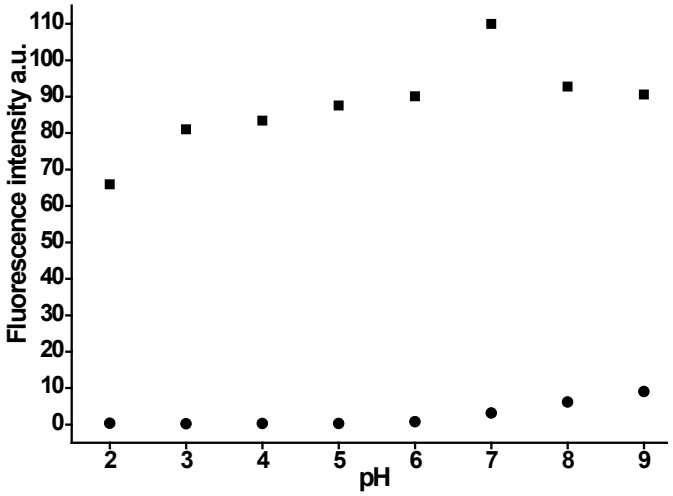

Figure SI-5. Fluorescence intensity at 514 nm (upon excitation at 450 nm) of HEPES (7 mM, pH 7.4)-DMSO 99:1 v/v solutions of chemodosimeter **1** (5.0 μM) at different pH values in absence (●) and in the presence of 10 equivalents of HS^- anion (■).

Hydrolysis of probe 1 with HS^- anion

Probe **1** (50 mg, 0.16 mmol) was dissolved in ethanol (5 mL) and then an excess of Na_2S (200 mg, 0.83 mmol) was added. The reaction was stirred at room temperature for 3 h. After that, the ethanol was eliminated in a rotary evaporator and the crude obtained was purified by silica column chromatography with hexane-acetone 1:1 v/v as eluent. The ^1H and ^{13}C of the final product (21.8 mg, 0.15 mmol, 94% yield) was coincident with that of 8-hydroxyquinoline.

Cell culture conditions

HeLa human cervix adenocarcinoma cells were purchased from the German Resource Centre for Biological Materials (DSMZ) and were grown in DMEM supplemented with 10% FBS. Cells were maintained at 37 ºC in an atmosphere of 5% carbon dioxide and 95% air and underwent passage twice a week.

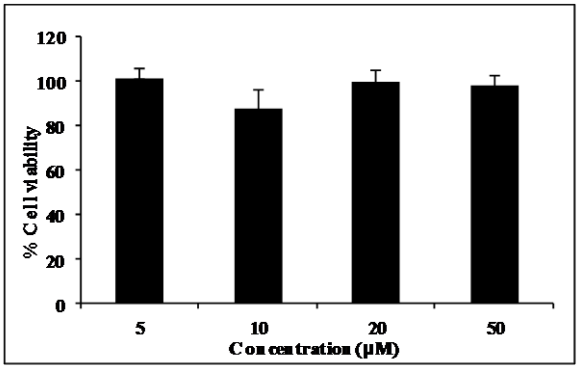

Figure SI-6. Cell viability studies with **1**. HeLa cells were treated with chemodosimeter **1** at concentrations of 5, 10, 20 and 50 μM for 24 hours. Cell viability was quantified by means of WST-1 assay. Three independent experiments were performed and data are reported as (mean ± SE).

WST-1 cell viability assay

HeLa cells were seeded in a 96-well plate at a density of 2.5 x 10^3 cells/well in 100 μL of DMEM and were incubated 24 hours in a CO_2 incubator at 37 ºC. Then, probe **1** in DMSO were added to cells in sextuplicate at final concentrations of 5, 10, 20 and 50 μM. After 23 hours, 7 μL of WST-1 were added to each well and then incubated for 1 hour more. Before reading the plate, it was shaked for one minute

to ensure homogeneous distribution of colour. Then the absorbance was measured at a wavelength of 450 nm. The results are depicted in figure SI-6.

Live Confocal Microscopy

HeLa cells were seeded in a 24 mm Ø glass coverslips in six-well plates at a seeding density of 1 x 10^5 cells /well. After 24 hours, cells were treated with chemodosimeter **1** for 30 minutes at a final concentration of 10 μM. Then the medium was changed and the well was washed with PBS. After that, a solution of Na_2S in PBS was added at a final concentration of 0, 200 and 500 μM for 1 hour. Then, coverslips were washed twice to eliminate compounds and, were visualized under a confocal microscope employing Leica TCS SP2 AOBS (Leica Microsystems Heidelberg GmbH, Mannheim, Germany) inverted laser scanning confocal microscope using oil objectives: 63X Plan-Apochromat-Lambda Blue 1.4 N.A. Confocal microscopy studies were performed by Confocal Microscopy Service (CIPF). The images were acquired with an excitation wavelength of 450 nm (argon laser) and a emission wavelength of 470-570 nm. Two-dimensional pseudo colour images (255 colour levels) were gathered with a size of 1024 x 1024 pixels and Airy 1 pinhole diameter. All confocal images were acquired using the same settings and the distribution of fluorescence was analyzed using the Image J Software. Three independent experiments were performed obtaining similar results.