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Supplementary Information

Quinoline-based fluorescence probe for the detection and monitoring of hypochlorous acid in rheumatoid arthritis model

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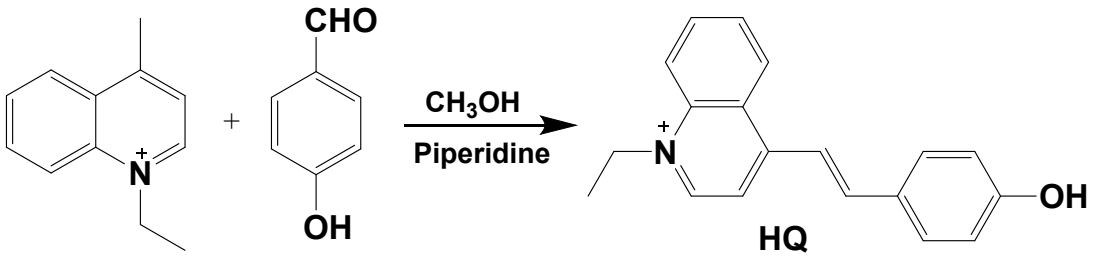
Materials and Instruments

4-Methylquinoline and 4-hydroxybenzaldehyde were purchased from Macklin reagent Co. (Shanghai, China). Iodoethane was obtained from *J&K* (Beijing, China). Piperidine, metal ions (nitrate salts), anions (sodium salts) and sodium hypochlorite (NaOCl) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Chromatography plates were purchased from commercial sources with specifications of 25 mm×75 mm and 0.20–0.25 mm thickness. All the experimental live zebrafish were obtained from the Experimental Animal Center of Dalian Medical University and the experiments were performed in compliance with the relevant laws and guidelines. All solvents and reagents were of analytical grade from commercial suppliers and were used without further purification.

^{1}HNMR and ^{13}CNMR spectra were recorded with an AVANCE 400MHZ spectrometer (BRUKER) with chemical shifts reported as *ppm* (in DMSO-d_6, TMS as internal standard). Coupling constants (*J* values) are reported in hertz. API mass spectra were recorded on an Agilent 6530 QTOF spectrometer. Absorption spectra were measured with a Perkin Elmer Lambda 900 UV/VIS/NIR spectrophotometer (USA). Fluorescence spectra were measured with Perkin Elmer LS55 luminescence spectrometer (USA). All pH measurements were made with an OHAUS Starter 3100/f meter (USA). HOCl in adult zebrafish and mice were imaged by a SPECTRAL Ami Imaging Systems (Spectral Instruments Imaging, LLC, Tucson, AZ) with an excitation filter 465 nm and an emission filter 570 nm. Ami-view Analysis software (Version 1.7.06) were used to calculate fluorescence intensity on region of interest (ROI), and values are presented as the mean±SD for each group of three experiments.

General procedures of spectra detection

A stock solution of probe **HQ** was prepared in dimethyl sulfoxide (DMSO) at the concentration of 0.5 mM. Prior to spectroscopic measurements, the solution was freshly prepared by diluting the stock solution to PBS buffer solution at the final concentration of 10 μM (DMSO: H_2O=1:9, 20 mM, pH=7.4). Solutions of a series of anions and biomolecules (20 mM) were freshly prepared by dissolving corresponding chemicals in deionized water. A stock solution of HOCl was prepared by dilution of the commercial sodium hypochlorite solution and stored according to the previous literatures.[1] The concentration of HOCl was determined by using its molar extinction coefficient of 391 M^{-1} cm^{-1} at 292 nm before use. Hydroxyl radical (·OH) was generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide.[2] Superoxide anion radical (O_2^-) was prepared from KO_2 in DMSO. Singlet oxygen ($^1O_2$) was generated from the Na_2MoO_4-H_2O_2 system in 0.05 M carbonate buffer of pH 10.5.[3] $ONOO^-$ was obtained by using SINA-1 as a donor. Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30% solution, and was assayed using its molar absorption coefficient of 43.6 $M^{-1}\cdot cm^{-1}$ at 240 nm.[4] For spectroscopic analysis, HOCl at different concentrations was added into the probe **HQ** solution (total volume 3 mL), followed by the spectroscopic measurements immediately. Excitation and emission slits are 5 nm, 20 nm, respectively.



Scheme S1 The synthesis routine of fluorescence probe **HQ**.

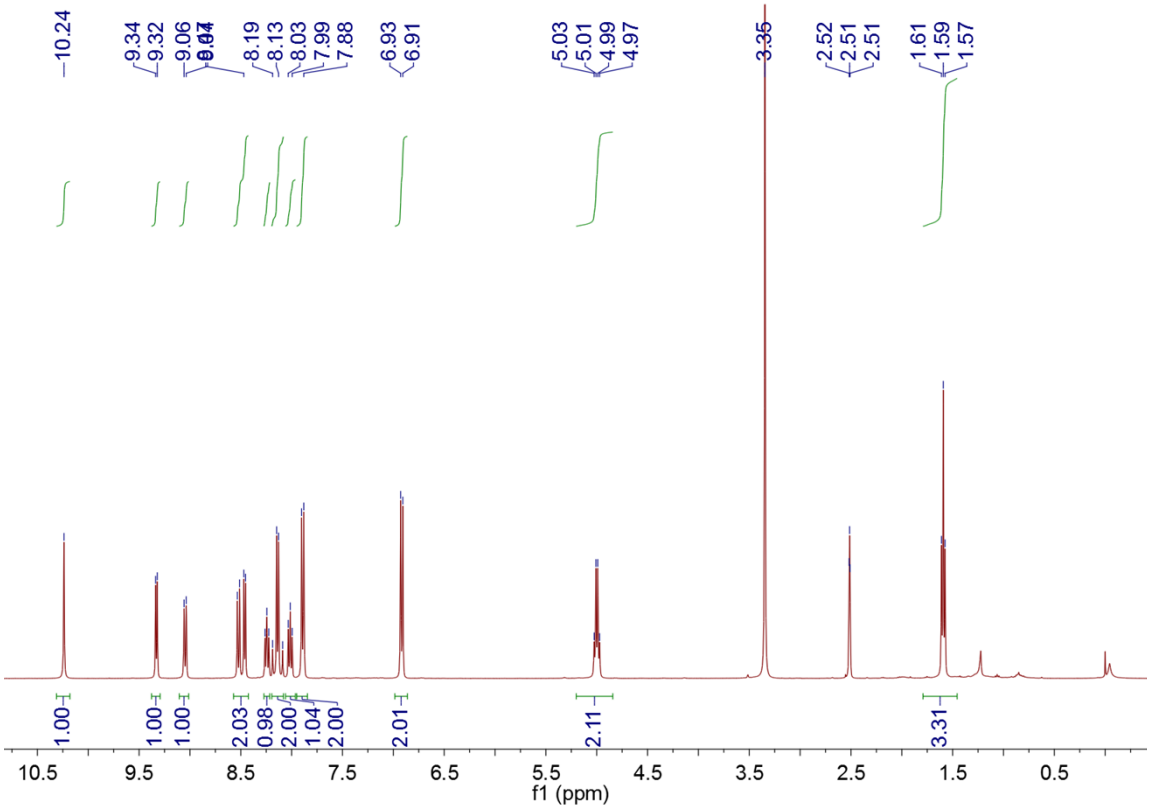


Fig. S1 ^{1}H NMR of **HQ** (DMSO-d_6, 400 MHz).

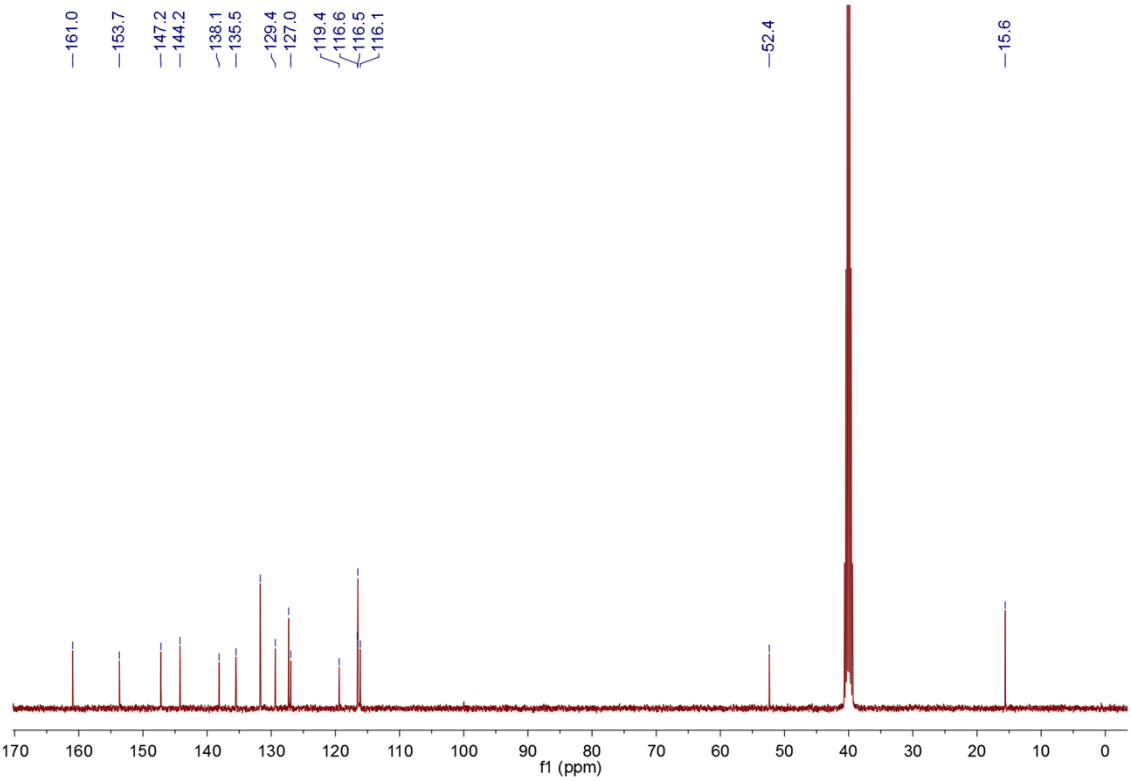


Fig. S2 ^{13}C NMR of **HQ** (DMSO-d_6, 101 MHz).

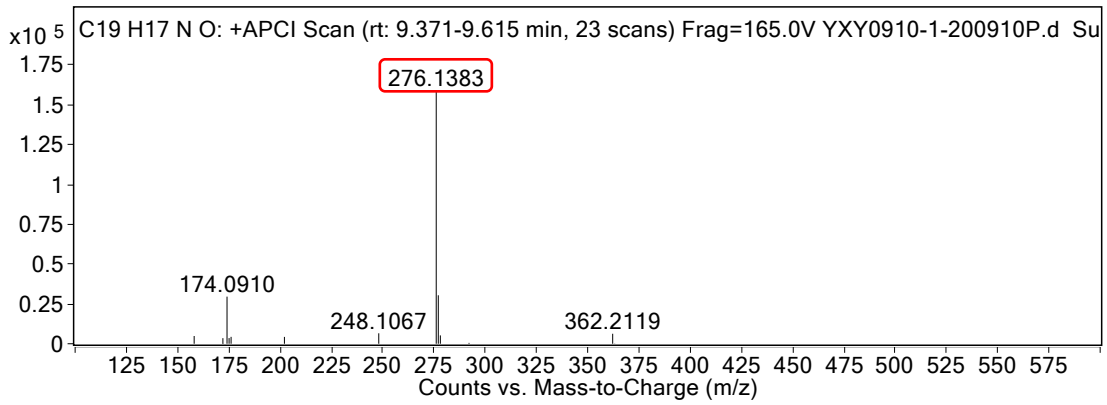


Fig. S3 HRMS of **HQ**.

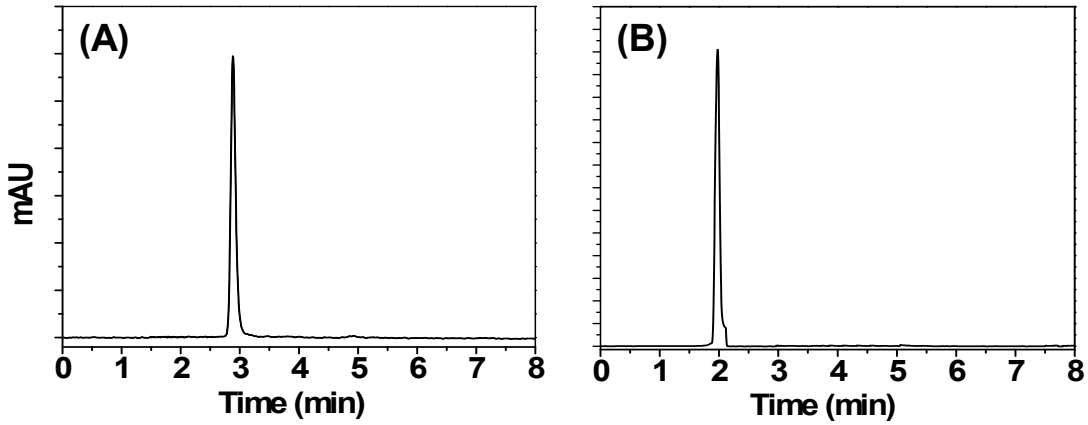


Fig. S4 HPLC of **HQ** (A) in the absence and (B) in the presence of HOCl.

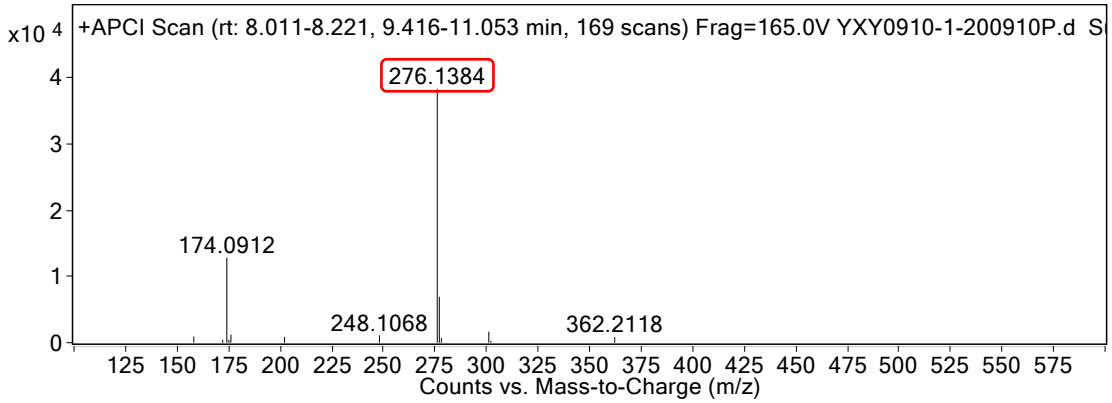


Fig. S5 HRMS of **HQ** in the presence of HOCl.

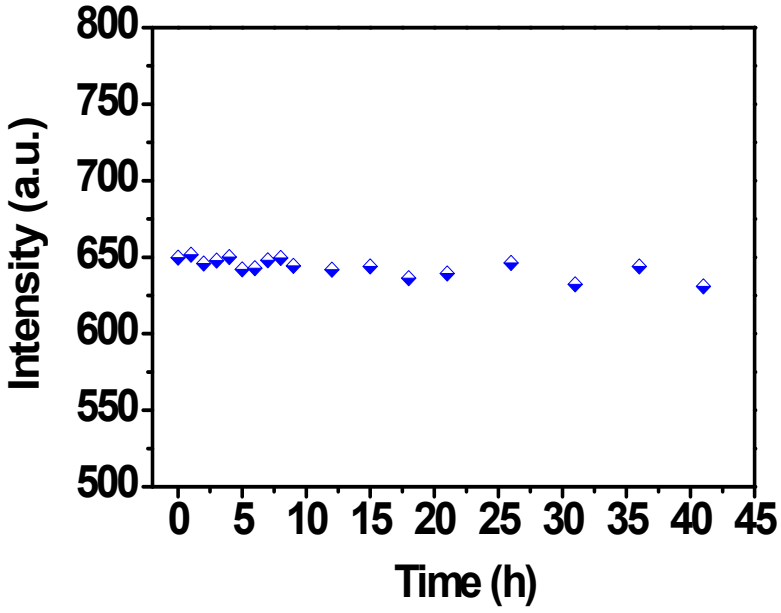


Fig. S6 Fluorescence changes of **HQ** (10 μM) in PBS aqueous buffer (DMSO: H_2O=1:9, 20 mM, pH=7.4). Excitation was performed at 450 nm, and emission was collected at 550 nm.

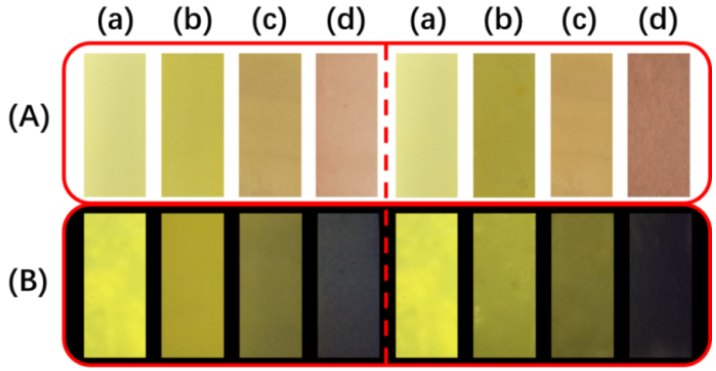


Fig. S7 Photographs of the (A) colorimetric and (B) fluormetric responses of the **HQ**-based chromatography plates upon exposure to different concentrations of HOCl in drinking water (left 4 groups) and tap water (right 4 groups): (a) 0 mM, (b) 0.1 mM, (c) 0.5 mM and (d) 1.0 mM.

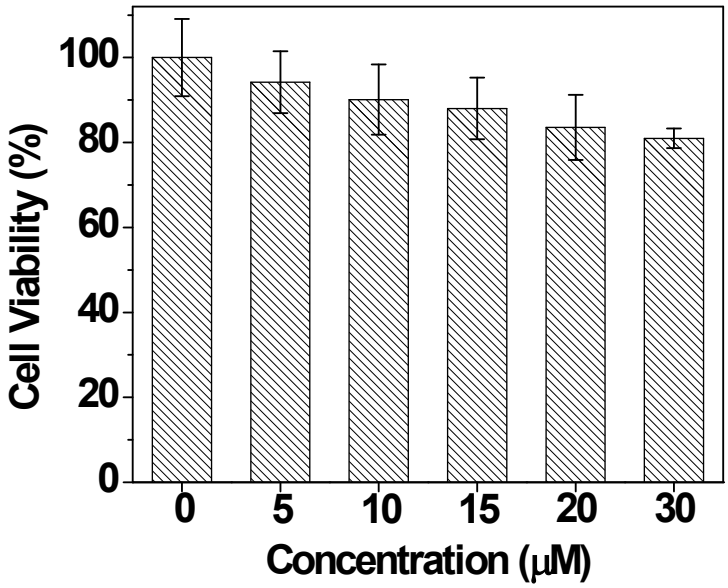


Fig. S8 The viability of A549 cells incubated with **HQ** (0-30 μM) for 24 h.

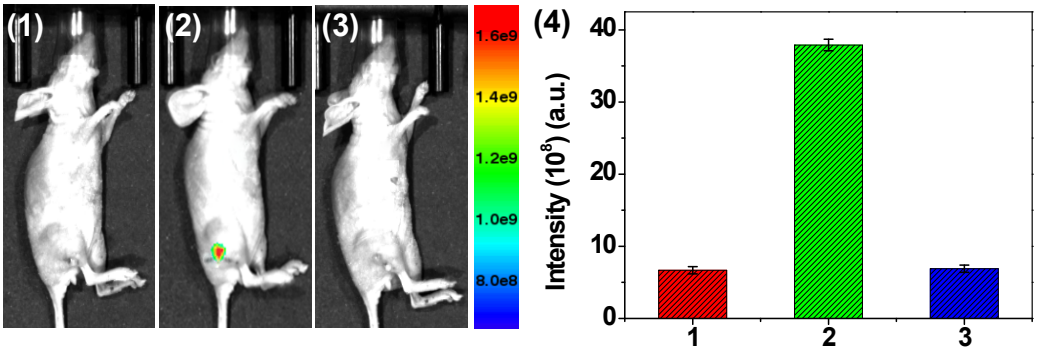


Fig. S9 Fluorescence imaging of exogenous HOCl using **HQ** in live nude mice. (1) Control group (mice only), (2) the leg was given a skin-pop injection of probe **HQ** (20 μM, 125 μL), (3) HOCl (200 μM, 80 μL) was injected into the same area of the leg. (4) Mean fluorescence intensity analysis of the mouse at different times shown in (1–3). Nude mice were imaged using an excitation filter (465 nm) and emission filter (570 nm).

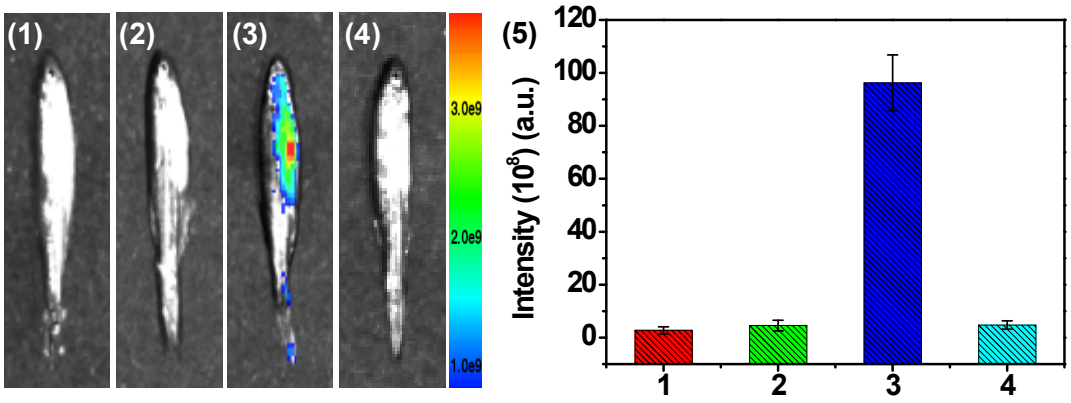


Fig.S10 Fluorescence imaging of endogenous HOCl production in zebrafish by using **HQ**. (1) zebrafish only, (2) zebrafish was stimulated with LPS (2 μg mL^{-1}) for 3 h, (3) zebrafish stained with **HQ** (20 μM) only for 3 min, (4) zebrafish pretreated with LPS (2 μg mL^{-1}) for 3 h and then stained with **HQ** for 3 min, (5) The mean fluorescence intensities of the areas of interest at different times shown in (1-4). Zebrafish was imaged using an excitation filter (465 nm) and emission filter (570 nm).

References

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