Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information for:

Detection of Acetylcholinesterase and Butyrylcholinesterase In Vitro and In Vivo by A New Fluorescence Probe

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1. Apparatus and reagents

Apparatus. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Shimadzu LC-MS 2010A instrument (Kyoto, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker DMX-600 spectrometer in CF_3COOD. UV-vis absorption spectra were recorded on a Hitachi U-3010 spectrophotometer (Kyoto, Japan). Fluorescence spectra were obtained on a Hitachi F-7000 fluorescence spectrometer (Hitachi Limited Ltd., Japan) in 1 cm quartz cells. The fluorescence imaging of zebrafish was performed by a confocal laser scanning microscope (Leica, Germany). Electron distribution, optimized structures, and energy state of the HOMO and LUMO for probe **OHPD** and resorufin are calculated at M06-2X/6-31G (d,p) level.

Reagents. Resorufin, dimethylcarbamyl chloride, AChE and BChE were purchased from Sigma Aldrich Co. Ltd. The stock solution (1 mM) of probe **OHPD** was prepared by dissolving probe **OHPD** in deoxygenated dimethyl sulphoxide (DMSO). All the inorganic salts used in the experiment were purchased from Tianjin Tianli Chemical Reagent Co., Ltd. The 3-day-old zebrafish were obtained from obtained from Haibin Xia' group, college of life sciences, Shaanxi Normal University (Xi'an, China). Ultrapure water was purified by a Milli-Q reference system (Millipore).

2. Synthesis of probe OHPD

The method of synthesizing probe **OHPD** is described in Scheme 1. To a solution of resorufin (117.5 mg, 0.5 mmol) in acetonitrile (5 mL), K_2CO_3 (138 mg, 1 mmol) was added and the mixture was stirred for 20 min at room temperature, and then dimethylcarbamoyl chloride (200 μL) was added, the mixture was stirred at 50 ℃ for 3 h. Finally, the solvent was evaporated under reduced pressure, and the obtained crude product was purified by silica gel chromatography (CH_2Cl_2/MeOH as an eluent) to yield probe **OHPD** as an orange solid. The ^{1}H and ^{13}C NMR spectra of probe **OHPD** are given in Fig.S1 and S2, respectively. ^{1}H NMR (600 MHz, CF_3COOD) δ 8.47 (dd, J = 63.5, 9.3 Hz, 2H), 8.00 (t, J = 1.9 Hz, 1H), 7.92-7.70 (m, 2H), 7.50 (t, J = 1.8 Hz, 1H), 3.23 (d, J = 57.9 Hz, 6H). ^{13}C NMR (151 MHz, CF_3COOD) δ 180.00, 160.78, 153.94, 152.01, 146.39, 142.38, 137.06, 135.49,

133.65, 128.05, 124.40, 109.23, 102.55, 35.45. ESI-MS m/z: calculated for probe **OHPD** ($C_{15}H_{12}N_2O_4$, $[M]^+$), 284.08; found, 285.08 and 307.07 (Fig. S3).

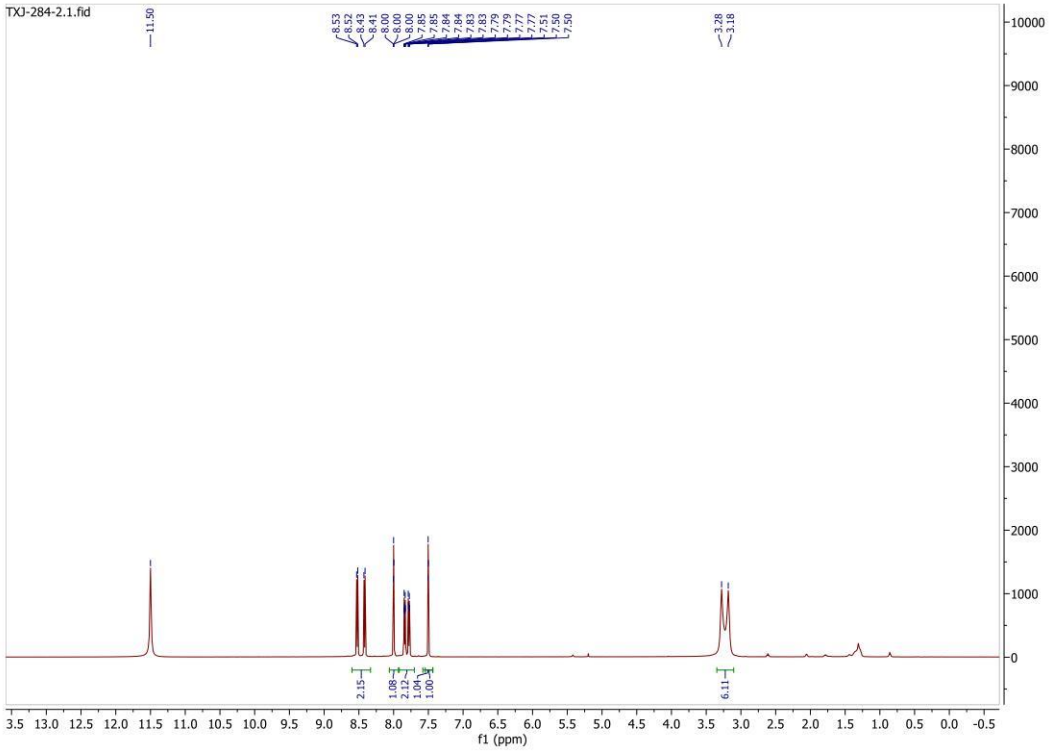

Fig. S1 ^{1}H NMR spectrum of probe **OHPD** in CF_3COOD.

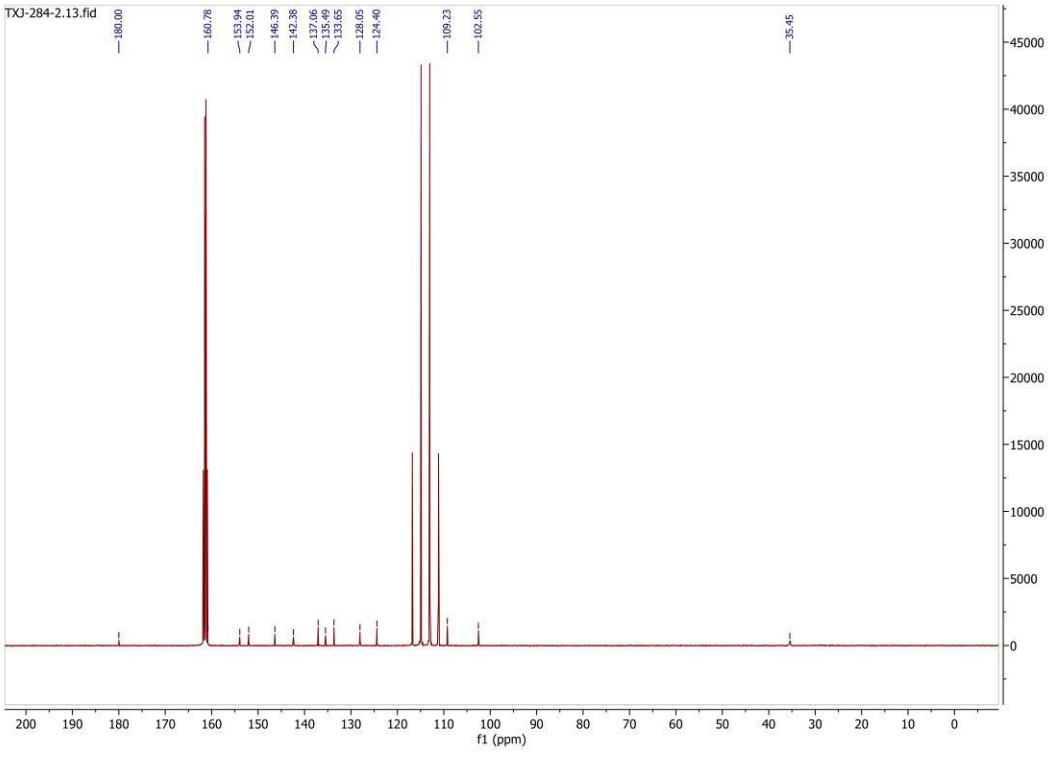

Fig. S2 ^{13}C NMR spectrum of probe **OHPD** in CF_3COOD.

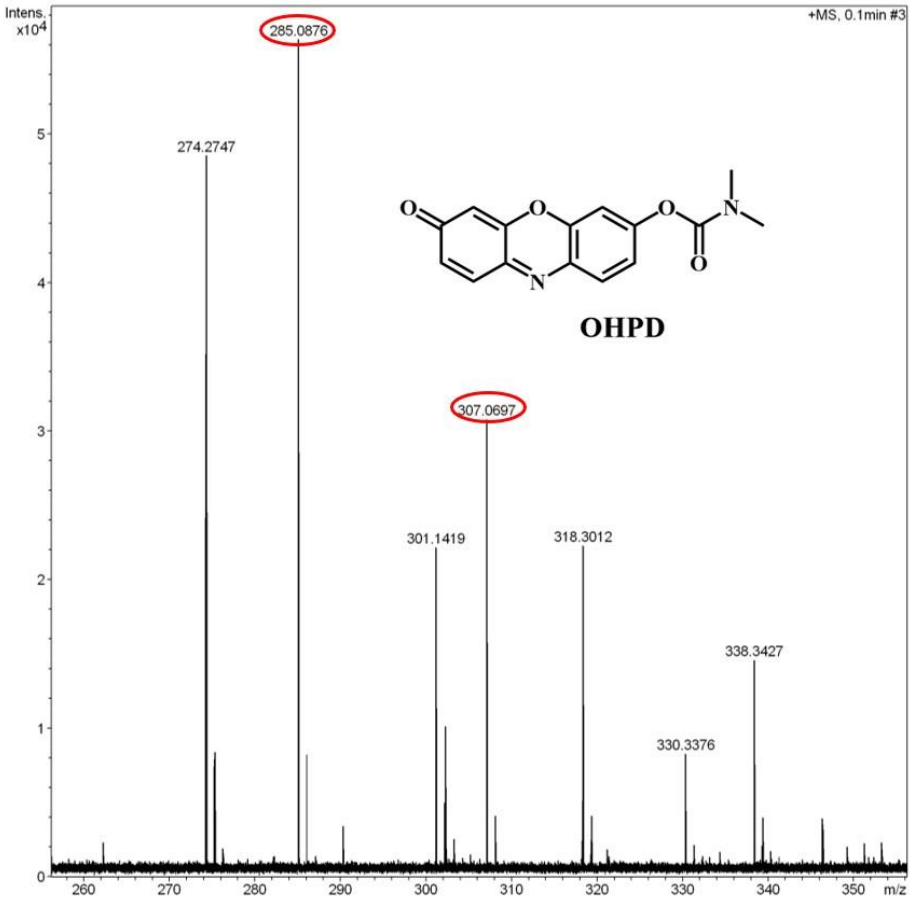

Fig. S3 Electrospray ionization mass spectrum of probe **OHPD**.

3. General procedure for AChE and BChE assay

All of the fluorescence measurements of probe **OHPD** (10 μM) and AChE/BChE were carried out in PBS (10 mM, pH 7.4). In a test tube, PBS (4 mL) and the stock solution (50 μL) of probe **OHPD** were mixed, and then various concentrations of AChE/BChE and other potential interfering substances were added to the mixture. The final volume was set to 5 mL with PBS. In addition, the final concentration of AChE/BChE were adjusted to 100 U/mL and 5 U/mL, respectively. Moreover, the fluorescence spectrum and absorption spectra were collected after incubation at 37 ℃ for 4 h in a shaker incubator.

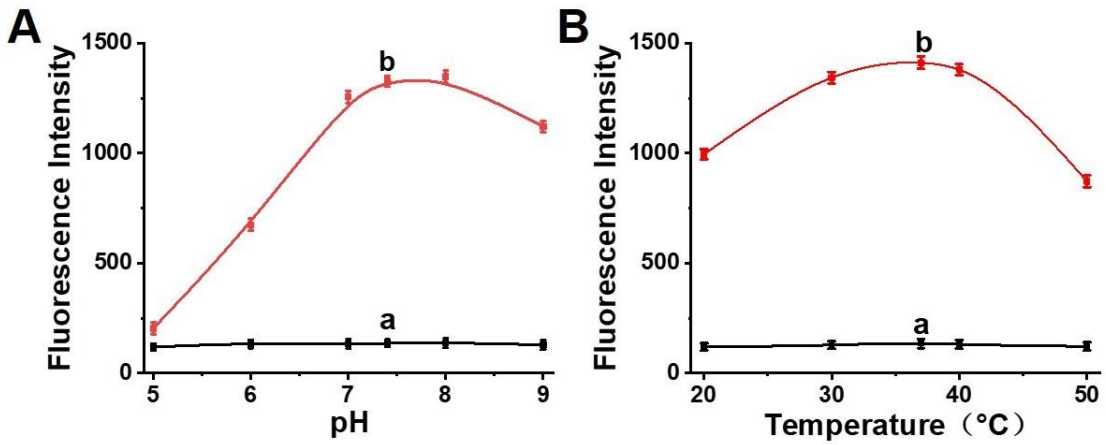

Fig. S4 Effects of (A) pH and (B) temperature on the fluorescence (λex = 550 nm) of probe **OHPD** (10 μM) reacting with AChE (100 U/mL). The results are the mean ± standard deviation of three separate measurements.

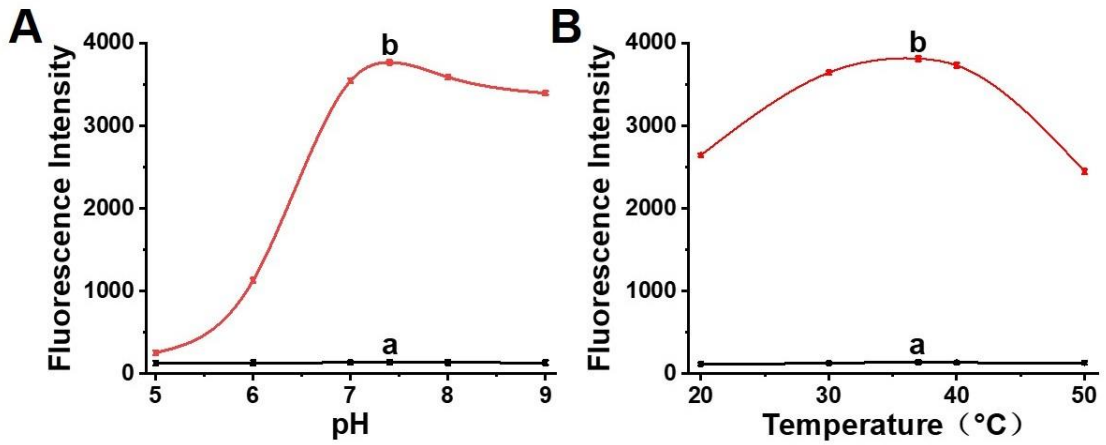

Fig. S5 Effects of (A) pH and (B) temperature on the fluorescence (λex = 550 nm) of probe OHPD (10 μM) reacting with BChE (5 U/mL). The results are the mean ± standard deviation of three separate measurements.

4. Lineweaver-Burke plot for ChEs-catalyzed reaction

By the Lineweaver-Burk plots, the rates of probe **OHPD** reacting with AChE and BChE were determined, respectively. Conditions: AChE (50 U/mL) react with different concentration (5 μM, 10 μM, 15 μM, 25 μM, 50 μM, 100 μM, and 200 μM) of probe **OHPD** in PBS buffer (10 mM, pH 7.40) at 37 ºC. BChE (5 U/mL) react with different concentration (0.05 μM, 0.1 μM, 0.25 μM, 0.5 μM, 1 μM, 5 μM, 10 μM, and 20 μM) of probe **OHPD** in PBS buffer (10 mM, pH 7.40) at 37 ºC.

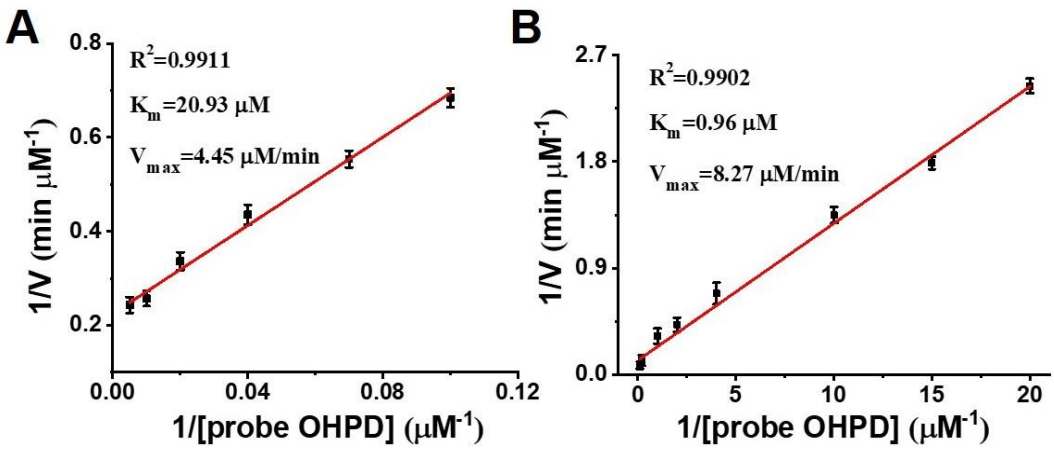

Fig. S6 Lineweaver-Burke plot for ChEs-catalyzed reaction of probe **OHPD**. (A) Lineweaver-Burke plot for AChE-catalyzed reaction of probe **OHPD**. (B) Lineweaver-Burke plot for BChE-catalyzed reaction of probe **OHPD**.

5. Theoretical calculation

We have carried out a series of theoretical calculations at the M06-2X/6-31G (d,p) level to better explain the proposed reaction mechanism, including the spatial structures, the active sites, the charge distribution, and the intermolecular interaction force. Moreover, we calculated the docking scores *via* Surflex docking-scoring combinations to assess the binding affinity of probe **OHPD** to AChE and BChE.

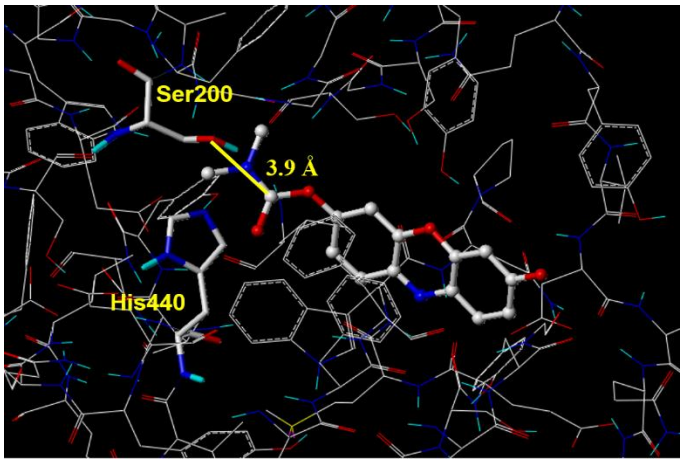

Fig. S7 The details of the binding of probe **OHPD** to AChE (calculated *via* Surflex docking-scoring combinations).

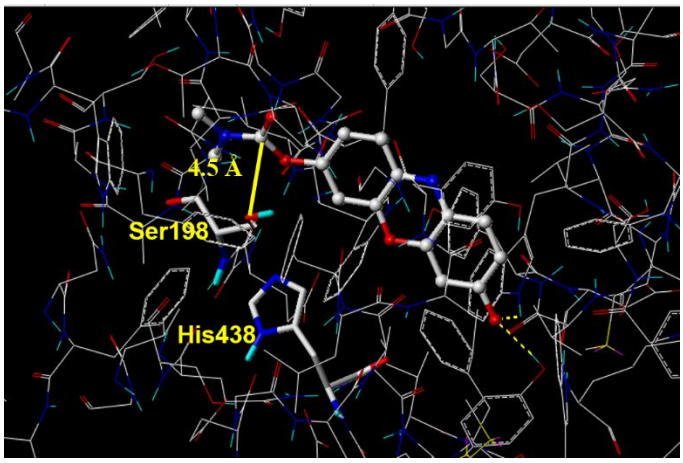

Fig. S8 The details of the binding of probe **OHPD** to BChE (calculated *via* Surflex docking-scoring combinations).

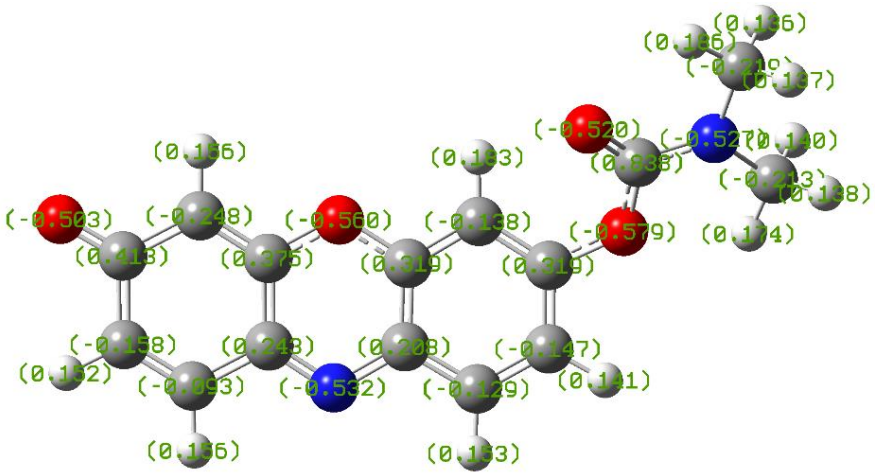

Fig. S9 Optimized structures and calculated atomic charges of probe **OHPD**.

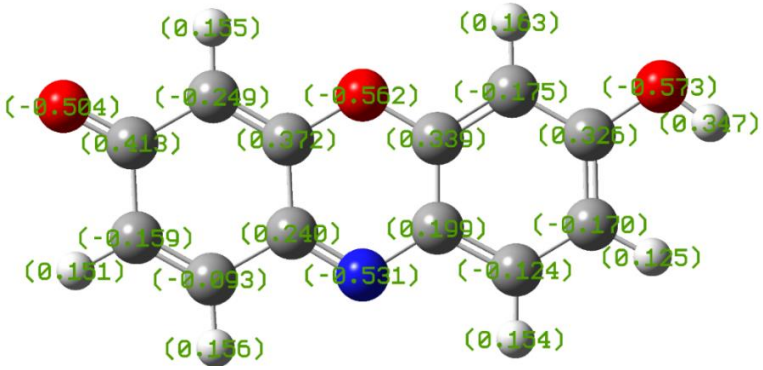

Fig. S10 Optimized structures and calculated atomic charges of resorufin.

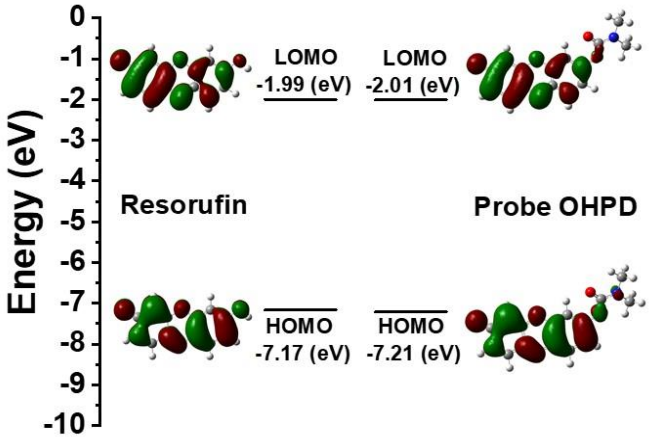

Fig. S11 Electron distribution and energy state of the HOMO and LUMO for resorufin and **OHPD**.

6. The cytotoxicity assay.

HeLa cells were seeded in 96-well U-bottom plates at a density of 7000 cells per well, and incubated with probe **OHPD** at varied concentrations (1-20 μM) at 37 °C for 24 h. Then, the culture media were discarded, and 0.1 mL of the MTT solution (0.5 mg/mL in DMEM) was added to each well, followed by incubation at 37 °C for 4 h. After shaking the plates for 10 min, absorbance values of the wells were read with a microplate reader at 490 nm.

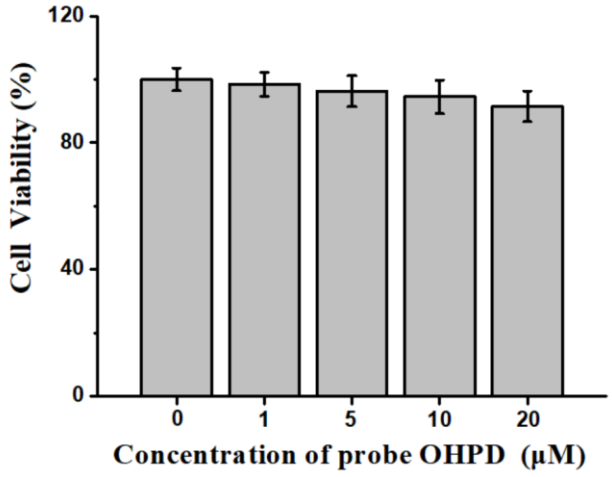

Fig. S12 Effects of probe **OHPD** with varied concentrations (0, 1, 5, 10 and 20 μM) on the viability of HeLa cells. The viability of HeLa cells without probe **OHPD** were defined as 100%. The results are the mean ± standard deviation of six separate measurements.

7. Fluorescence imaging in zebrafish

3-day-old zebrafish were cultured in E3 embryo media and were divided into three groups. Zebrafish in group (a) served as the control group, treated with probe **OHPD** (20 μM) only for 4 h, Zebrafish in group (b) were pretreated with donepezil (50 μM) for 2 h, and then incubated with probe **OHPD** (20 μM) for 4 h. Zebrafish in group (c) were pretreated with iso-OMPA (50 μM) for 2 h, and then treated with probe **OHPD** (20 μM) for 4 h. In addition, zebrafish were transferred into a glass slide before imaging, and the average pixel intensity of the zebrafish was measured using Image J software.

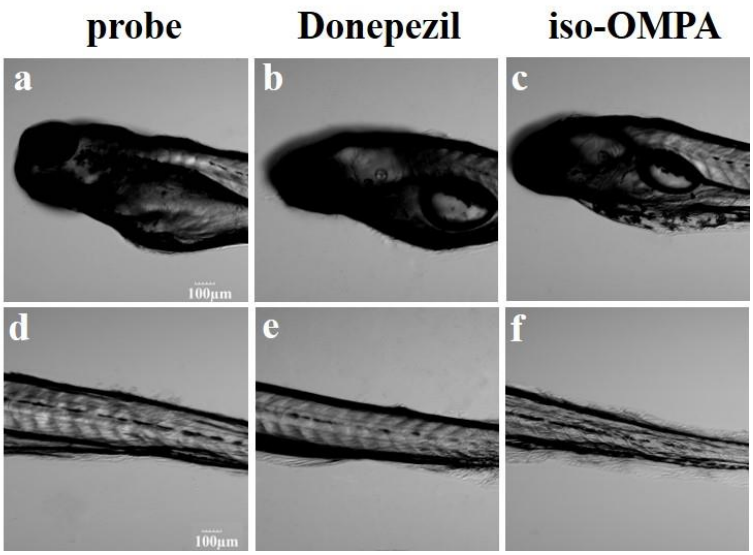

Fig. S13 The DIC images of the corresponding zebrafish in Fig. 4A. Scale bar = 100 μm.