# Electronic Supplementary Information for:

## A new metal-free near-infrared fluorescent probe based on nitrofuran for detection and bioimaging of carbon monoxide releasing molecule-2 in vivo

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## 1. Materials and chemicals

Electrospray ionization mass spectrometry (ESI-MS) was obtained using a Shimadzu LC-MS 2010A instrument. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker DMX-600 spectrometer in Methanol-d4. UV-vis absorption spectra were obtained using a Hitachi U-3010 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrometer in 1 cm quartz cells. A fluorescence spectrometer FLSP920 was employed to measure fluorescence quantum yields. And pH measurements were performed on a model HI-98128 pH meter. The 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny-ltetrazolium bromide (MTT) analysis was recorded on a microplate reader. A confocal laser scanning microscope (Leica) was utilized to perform fluorescence imaging of HeLa cells and zebrafish, mice were imaged by IVIS Spectrum imaging (Xengon).

IR-780 iodide, 2-(Bromomethyl)-5-nitrofuran, Tricarbonyldichlororuthenium (II) dimer (CORM-2) were purchased from Sigma-Aldrich Co. Ltd. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline buffer solution (PBS), penicillin and streptomycin were obtained from HyClone (South Logan, UT, U.S.A.). Stock solution (1 mM) of probe DXPN was prepared by dissolving a refined calculation of probe in deoxygenated dimethyl sulphoxide (DMSO). The silica gel (200-300 mesh) was used for the column chromatography and thin lay chromatography (TLC). The 3-day-old zebrafish were obtained from Haibin Xia' group, college of life sciences, Shaanxi Normal University (Xi'an, China). The 6-7 weeks old female BALB/c mice were obtained from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). Ultrapure water was purified by a Milli-Q reference system (Millipore).

## 2. Synthesis of probe DXPN

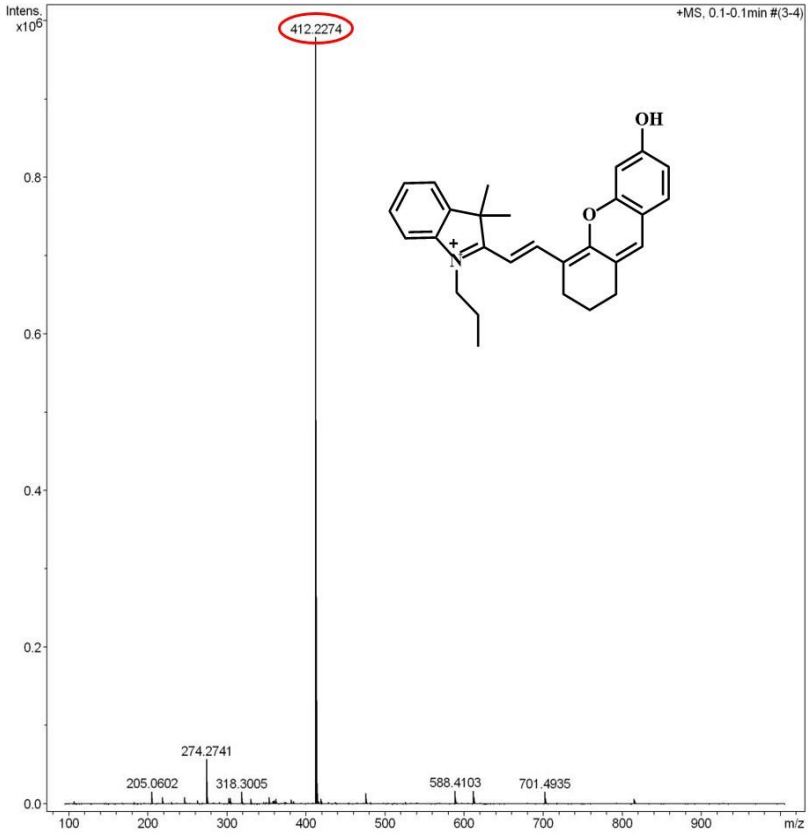


**Fig. S1** Electrospray ionization mass spectrum of fluorophore **DXPO**.

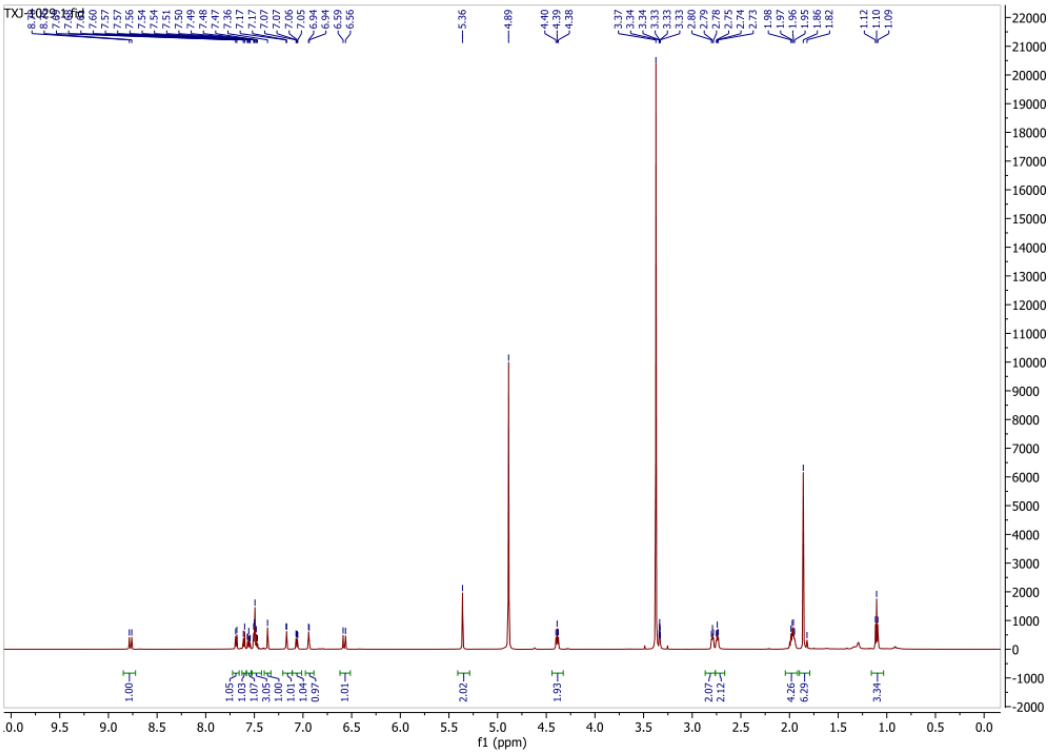


**Fig. S2** $^{1}$H NMR spectrum of probe **DXPN** in Methanol-$d_4$.

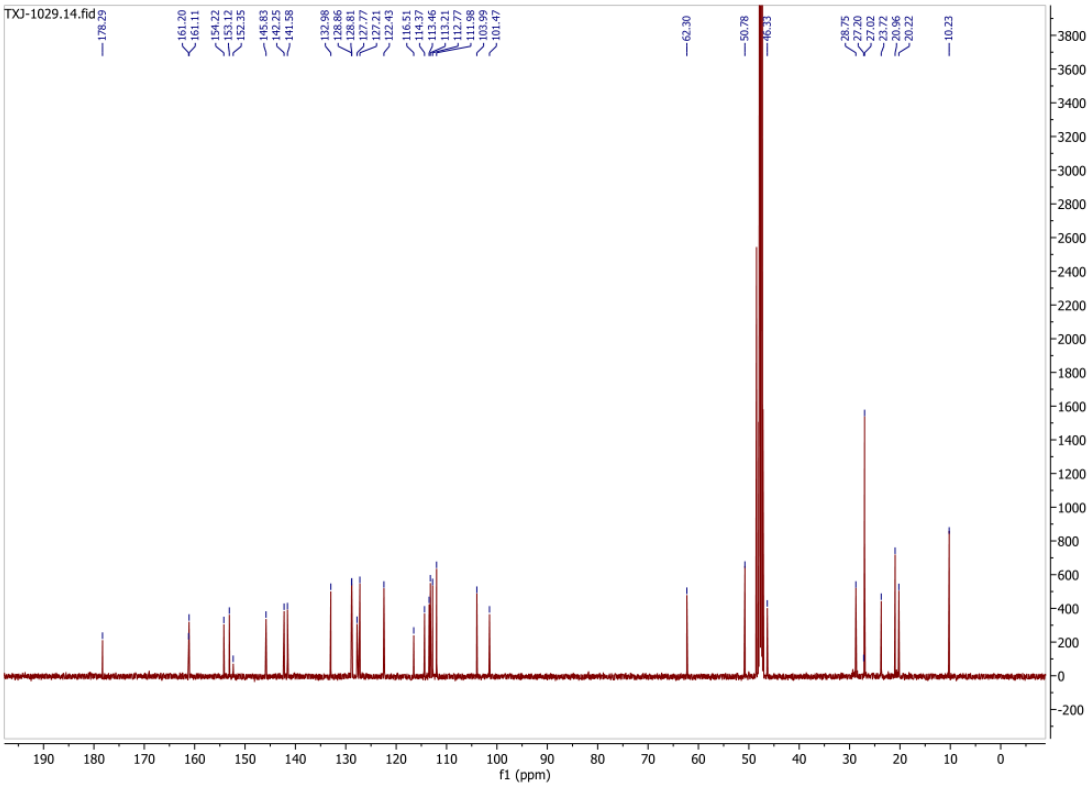


**Fig. S3** $^{13}$C NMR spectrum of probe **DXPN** in Methanol-$d_4$.

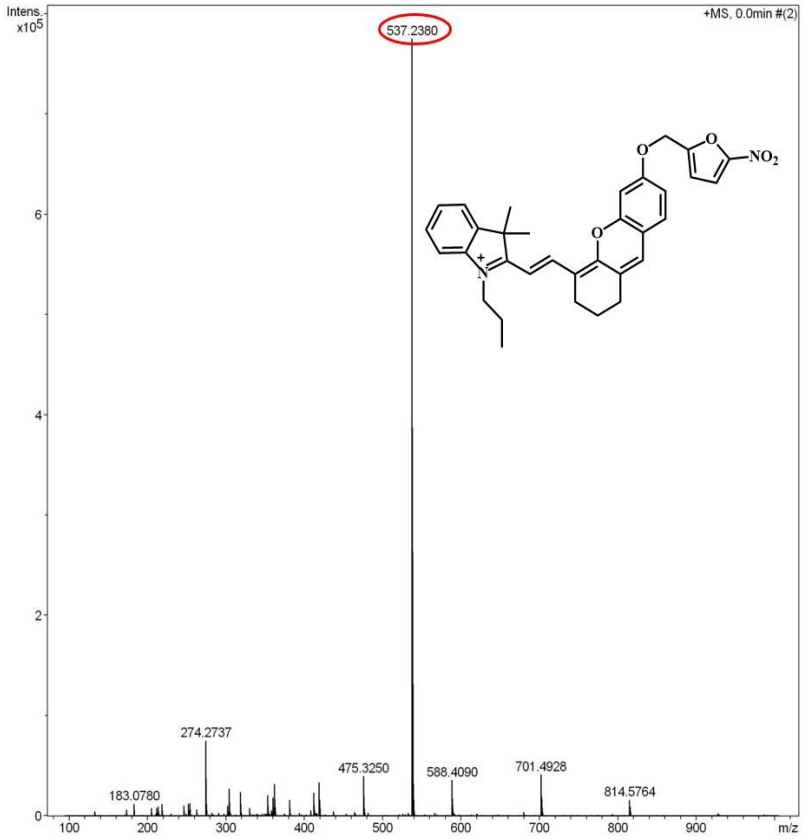


**Fig. S4** Electrospray ionization mass spectrum of probe **DXPN**.

## 3. Electrospray ionization mass spectrum of the reaction solution

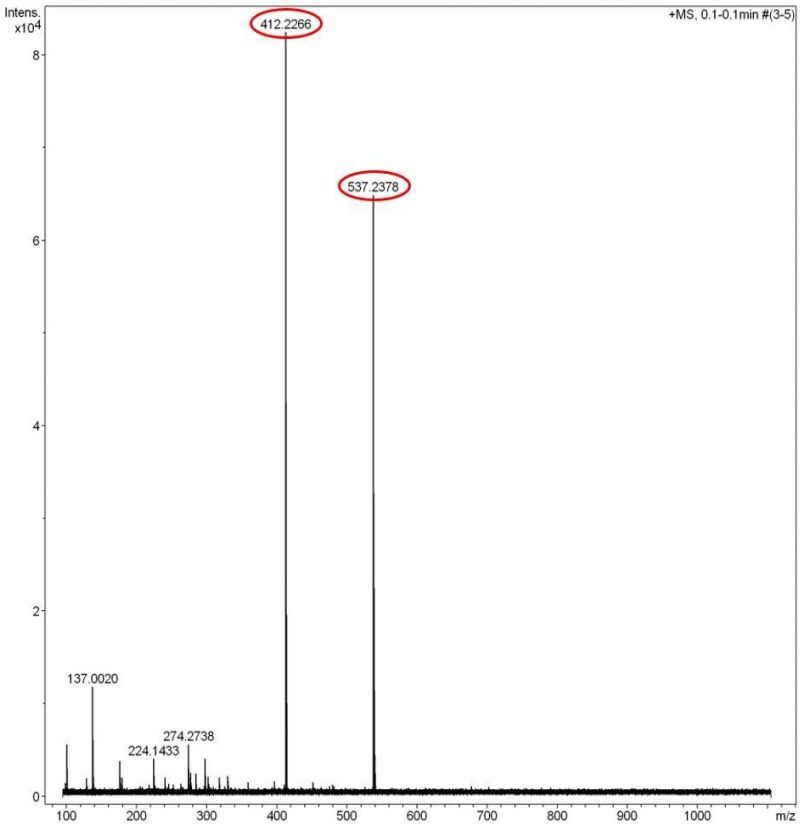


**Fig. S5** Electrospray ionization mass spectrum of the reaction solution.

## 4. General procedure for CORM-2 detection

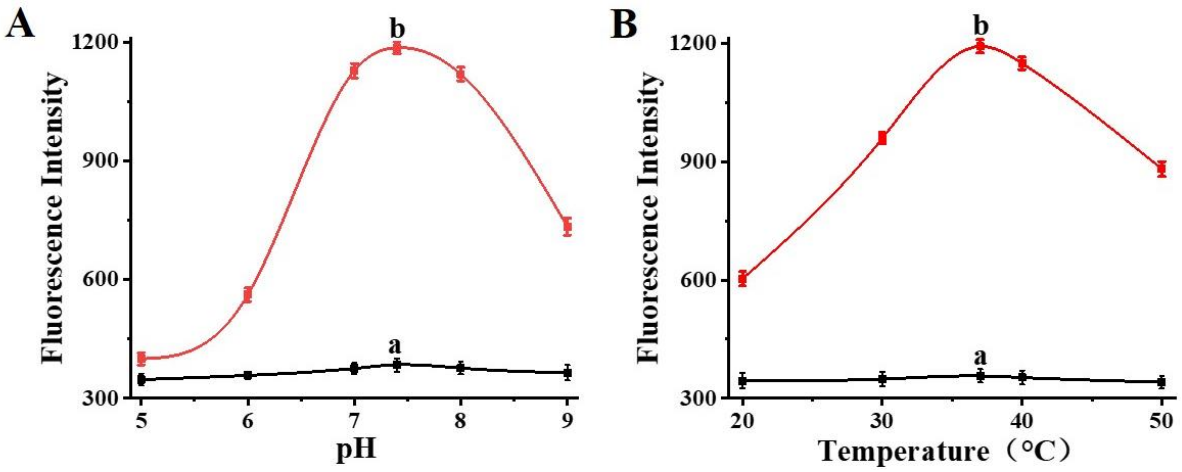


**Fig. S6** Dependence of fluorescence intensity (λex = 670 nm, λem = 712 nm) on pH (A) and temperature (B). Fluorescence spectra of probe **DXPN** (10 μM) before (a) and after (b) reaction with CORM-2 (100 μM). The reactions were performed at 37 °C for 40 min in 10 mM PBS (pH 7.4).

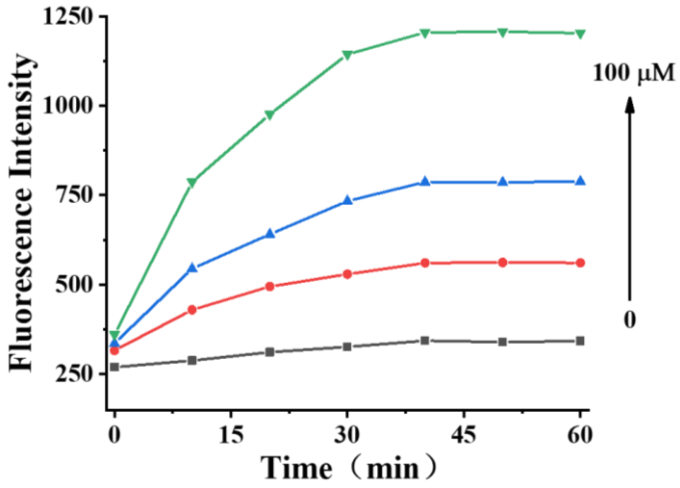


**Fig. S7** Dependence of fluorescence intensity (λex = 670 nm, λem = 712 nm) on the reaction time. Plots of fluorescence intensity of probe **DXPN** (10 μM) *vs.* the reaction time in the presence of varied concentrations of CORM-2 (from bottom to top): 0 (control), 25, 50, 100 μM.

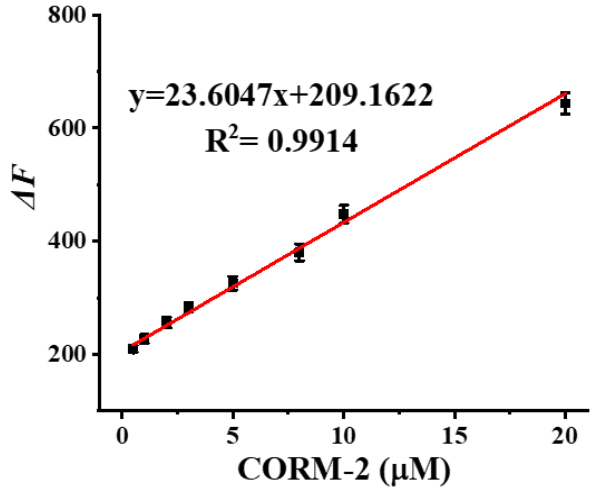


**Fig. S8** Dependence of the fluorescence intensity on the concentration of CORM-2. The plot of the fluorescence intensity against the CORM-2 concentration in the range of 0-100 μM. *ΔF* is the difference of fluorescence intensity of probe **DXPN** in the presence and absence of CORM-2. The measurements were performed in 10 mM PBS (pH 7.4) with $\lambda_{ex/em}$ = 670/712 nm.

## 5. Detection of CORM-2 based on paper chips

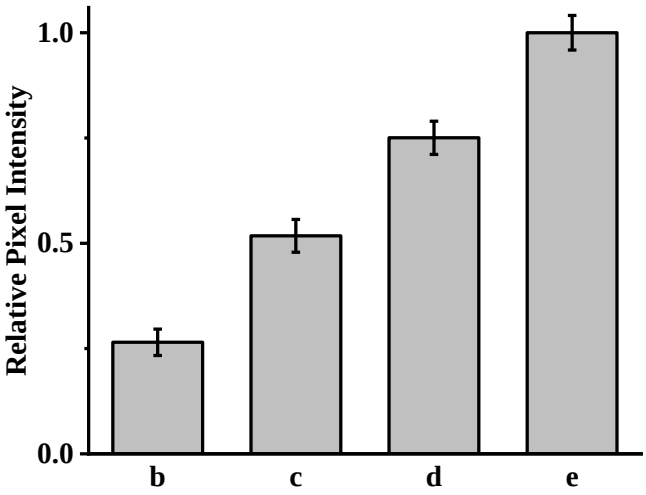


**Fig. S9** Relative pixel intensity measurements obtained from the images of paper chips. (b-e) paper chips preincubated with 25, 50, 75, 100 μM CORM-2 for 20 min and treated with probe **DXPN** (10 μM) for 40 min. The strongest fluorescence intensity from the image e is defined as 1.0. The results are the mean ± standard deviation of three separate measurements.

## 6. Cytotoxicity assay

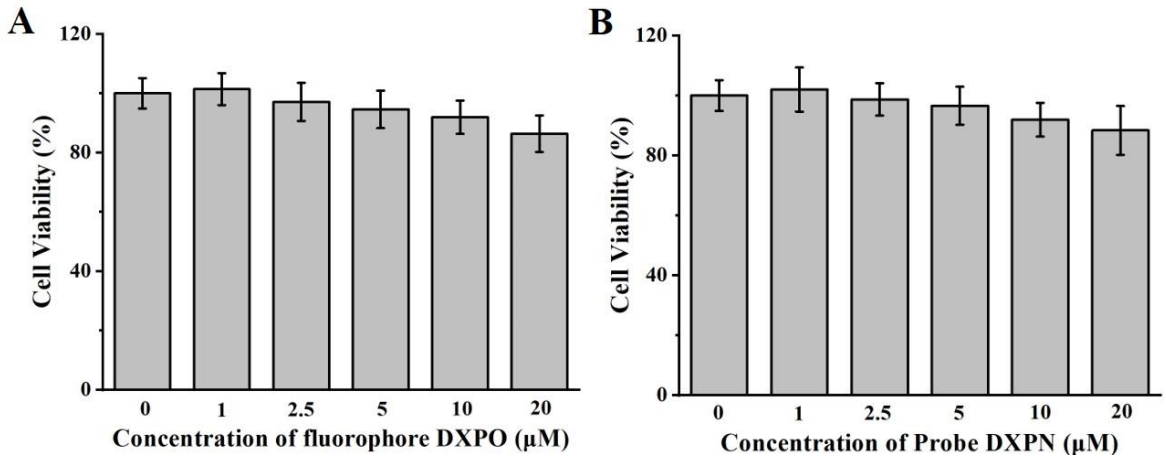


**Fig. S10** Effects of (A) fluorophore **DXPO** and (B) probe **DXPN** with varied concentrations (1-20 μM) on the viability of HeLa cells. The viability of the cells without probe **DXPN** or fluorophore DXPO is defined as 100%. The results are the mean ± standard deviation of six separate measurements.

## 7. Relative pixel intensity measurements in living cells

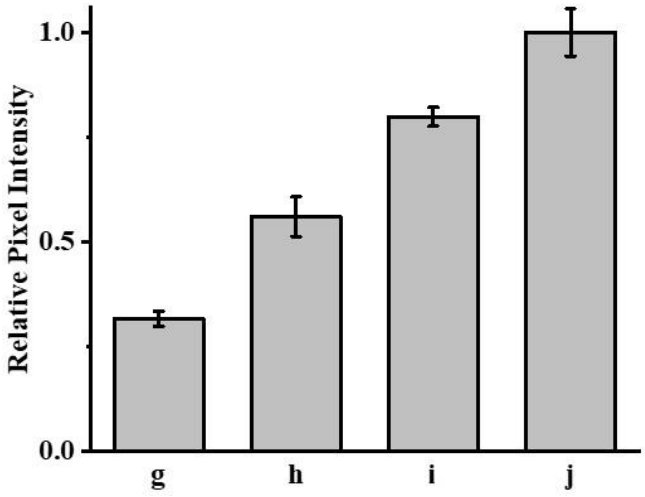


**Fig. S11** Relative pixel intensity measurements obtained from the images of HeLa cells. (g-j) cells preincubated with 25, 50, 75, 100 μM CORM-2 for 20 min and treated with probe **DXPN** (10 μM) for 40 min. The strongest fluorescence intensity from the image e is defined as 1.0. The results are the mean ± standard deviation of three separate measurements.

## 8. Fluorescence imaging in zebrafish

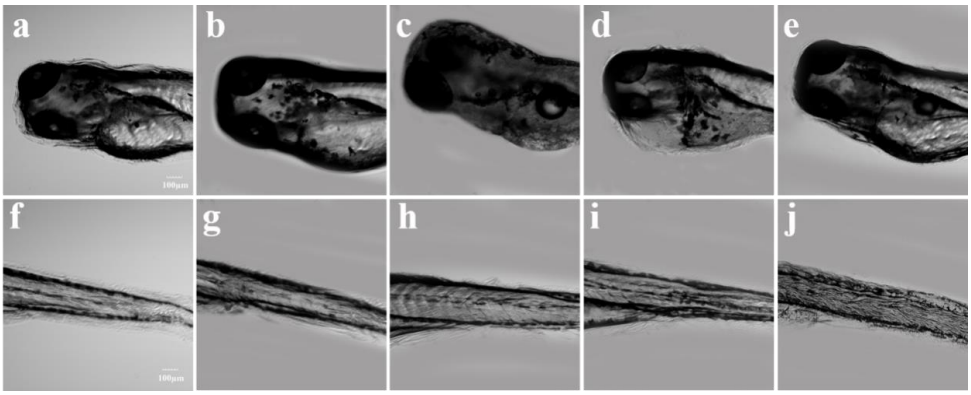


**Fig. S12** The DIC images of the corresponding samples in Fig. 3: (a-e and f-j) zebrafish preincubated with 0, 25, 50, 75, 100 μM CORM-2 for 20 min and then treated with probe **DXPN** (10 μM) for 40 min. Scale bar = 100 μm.

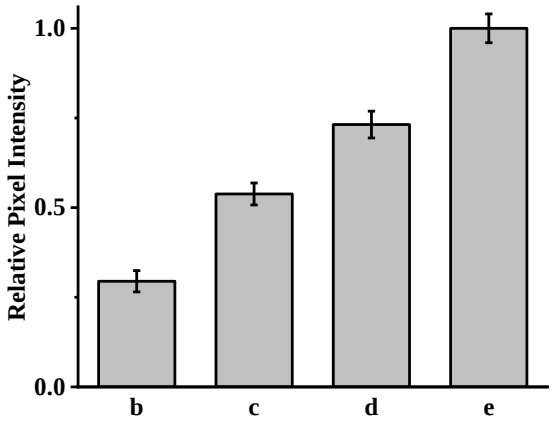


**Fig. S13** Relative pixel intensity measurements obtained from the images of zebrafish. (b-e and g-j) zebrafish preincubated with 25, 50, 75, 100 μM CORM-2 for 20 min and then treated with probe **DXPN** (10 μM) for 40 min. The strongest fluorescence intensity from the image e is defined as 1.0. The results are the mean ± standard deviation of three separate measurements. Scale bar = 100 μm.

## 9. Fluorescence imaging in mice

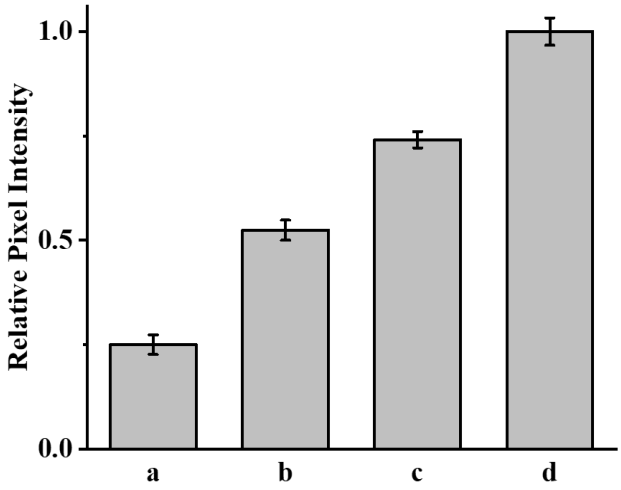


**Fig. S14** Relative pixel intensity measurements obtained from the images of mice after ip injection at 10 min (a), 20 min (b), 30 min (c), 40 min (d). The strongest fluorescence intensity of the mice after injection at 40 min is defined as 1.0. The results are the mean ± standard deviation of three separate measurements.

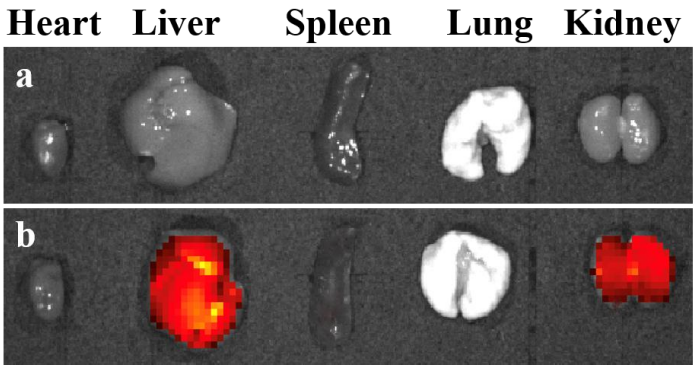


**Fig. S15** Fluorescence images of major organs (heart, liver, spleen, lung, and kidney) after 1 h injection.