Supporting Information

A simple turn-off fluorescent chemosensor based on Schiff base structure for ultrafast and highly selective trace detection of Cu^{2+} ions in aqueous solutions

Jing Wang [a], Lu Ren [b], Qiang Sun [a], Yanqi Liu [a], Wanru Jia [a], Huihong Zhang [a], Dawei Zhang [a]*

1.1 Calculation of the limit of detection (LOD) values

The LOD values were derived from fluorescence titration experiments based on a plot of fluorescence intensity and iron nitrate concentration:

$$\mathrm{LOD} = 3\sigma/k$$

Where σ is the standard deviation of the blank sample and k is the slope of the line of best fit.[1]

1.2 Cytotoxicity experiments

Living HepG-2 cells were provided by the School of Chemical and Pharmaceutical Engineering, Jilin Institute of Chemical Engineering. Inoculate cells overnight into a 96 well cell culture plate supplemented with 10% FBS (fetal bovine serum) in DMEM at 37 °C and 5% CO_2 atmosphere. Various concentrations (0, 10, 20, 30, 40，50 μM) of the probe DHP were added to the cell culture plate after the cells were washed with phosphate-buffered saline (PBS) three times. The cells were incubated overnight at 37 °C under a 5% CO_2 atmosphere. After incubation, the original medium was exchanged with new 100 μL 10 % FBS (fetal calf serum), followed by adding 10 μL MTT (0.5 mg/mL). After 4 h, the medium was removed, and 200 μL DMSO was added to each well. The absorbance at 570 nm was measured with a Spectramax microwell plate reader, and the cell viability towards the HepG-2 cells line were measured using the equation:[2]

Cell viability (%) = Mean absorbance (Treated cell) / Mean absorbance (Control cell)

1.3 Cell fluorescence imaging

During cell imagin experiments, the cells were divided into diverse groups and imaged after different treatments. HepG-2 cells were fixed in 24-well plates, washed with PBS, and then incubated in the dark for 10 min with the addition of MTT. The probes DHP and different concentrations of Cu^{2+} ions (0, 20, 40, 60, 80, 100 μM) were added and incubated for 30 min, respectively. The cells of each well were washed with 3 times PBS buffer after each step. The cells were eventually fixed on a circular slide and imaged by confocal electron fluorescence microscopy.[3,4]

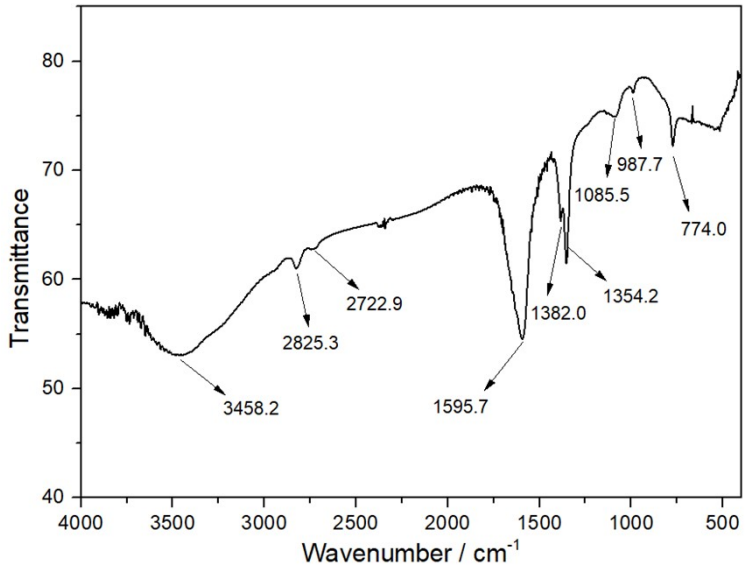

Fig. **S1** IR spectrum of DHP

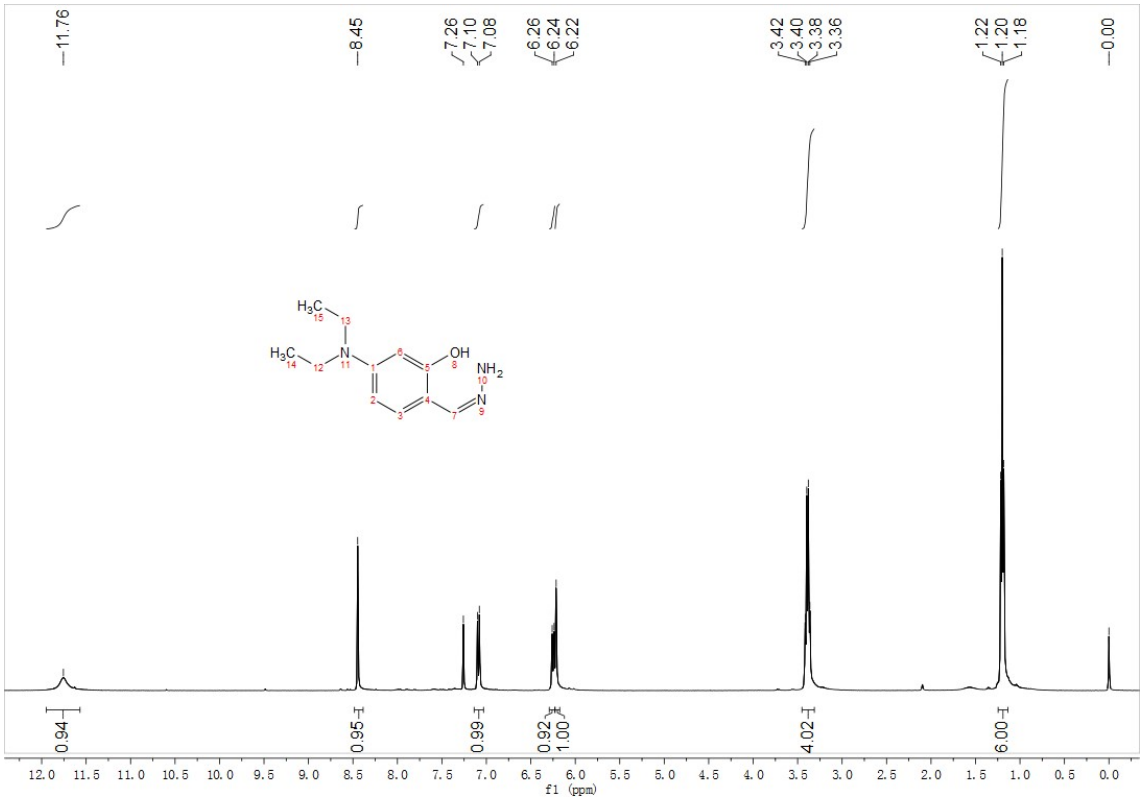

Fig. S2 ^{1}H NMR spectrum of DHP

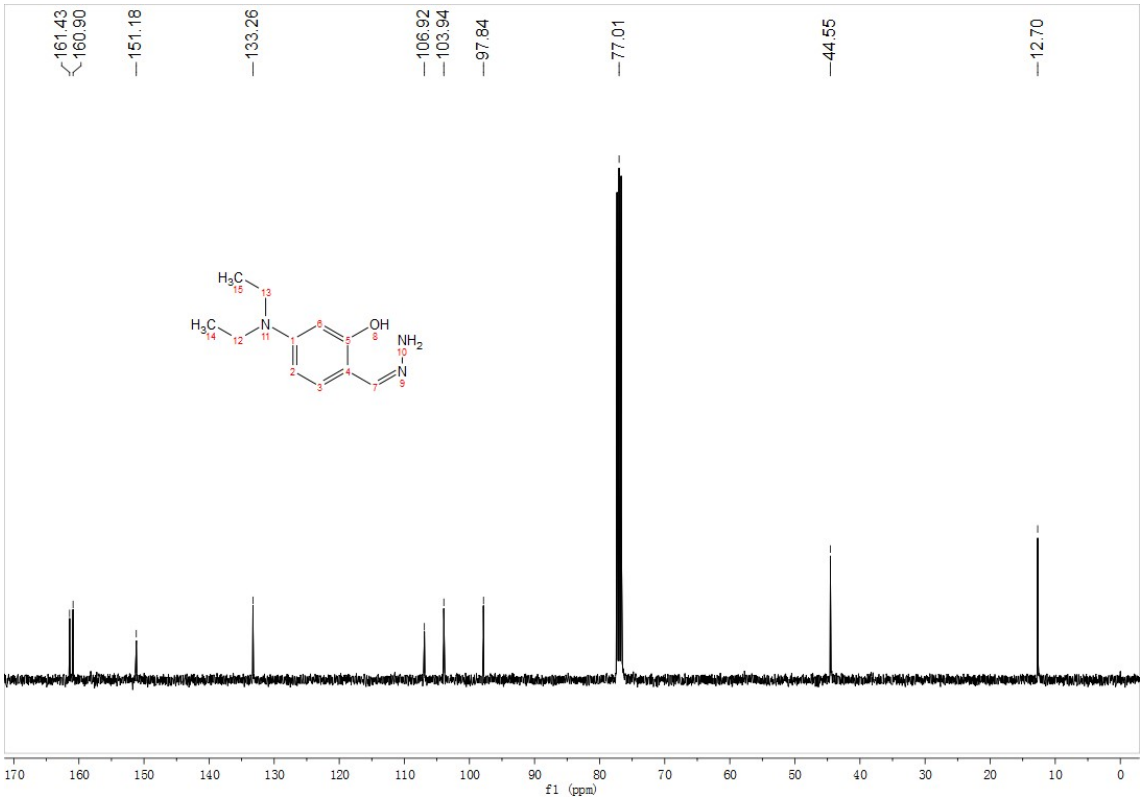

Fig. S3 ^{13}C NMR spectrum of DHP

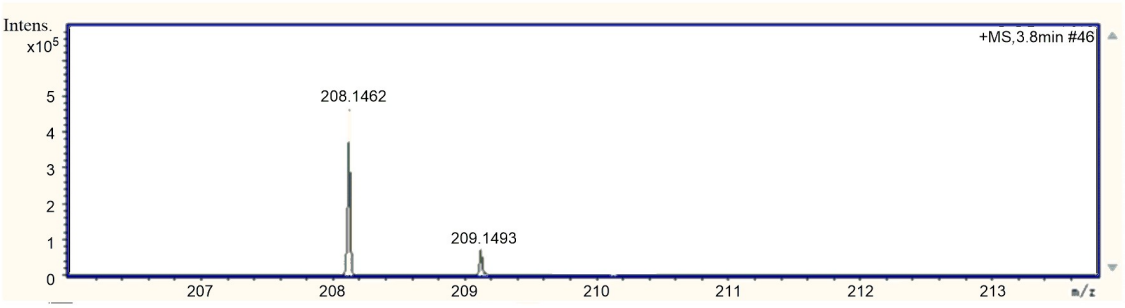

Fig. S4 HR-MS spectrum of DHP

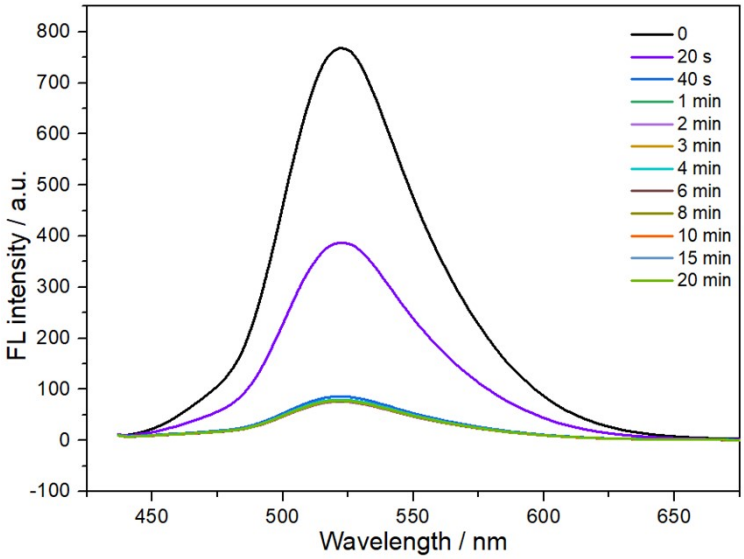

Fig. S5 Response time and stability of probe DHP (10 μM). Fluorescence intensity of DHP–Cu^{2+} (10 μM), EtOH/H_2O (1:1, v/v)

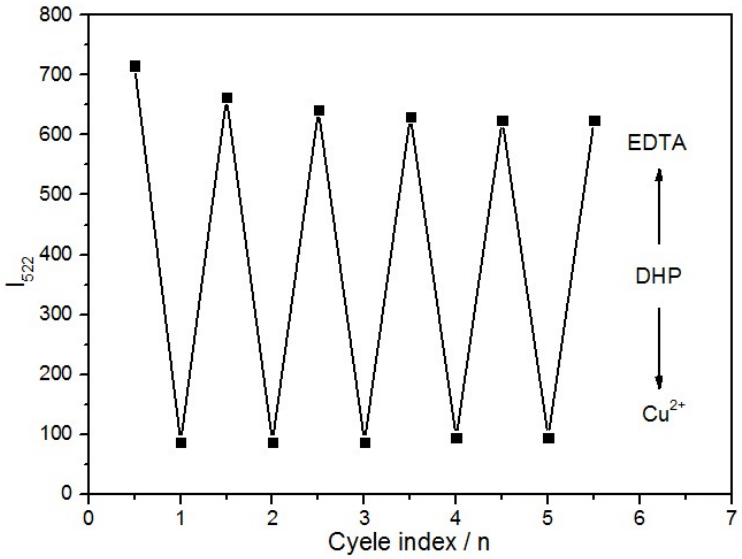

Fig. S6. The reversibility studies of DHP–Cu^{2+} with EDTA.

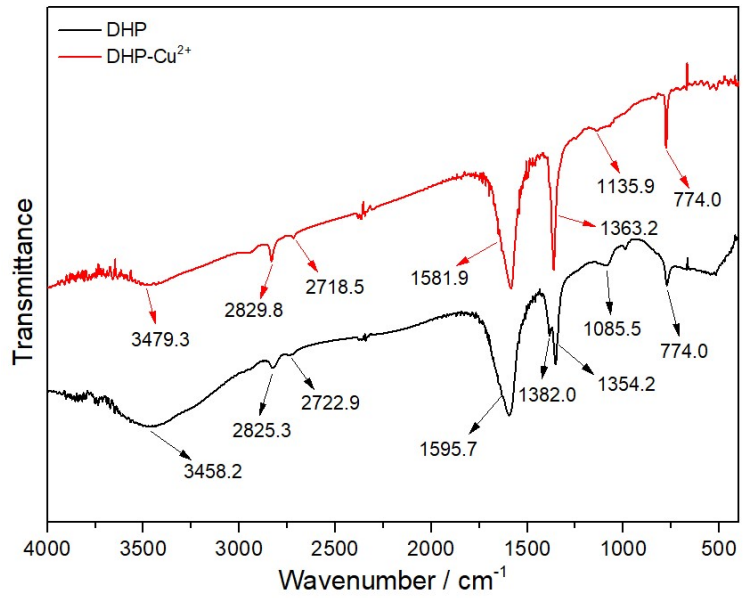

Fig. **S7** IR spectrum of DHP and DHP–Cu^{2+}

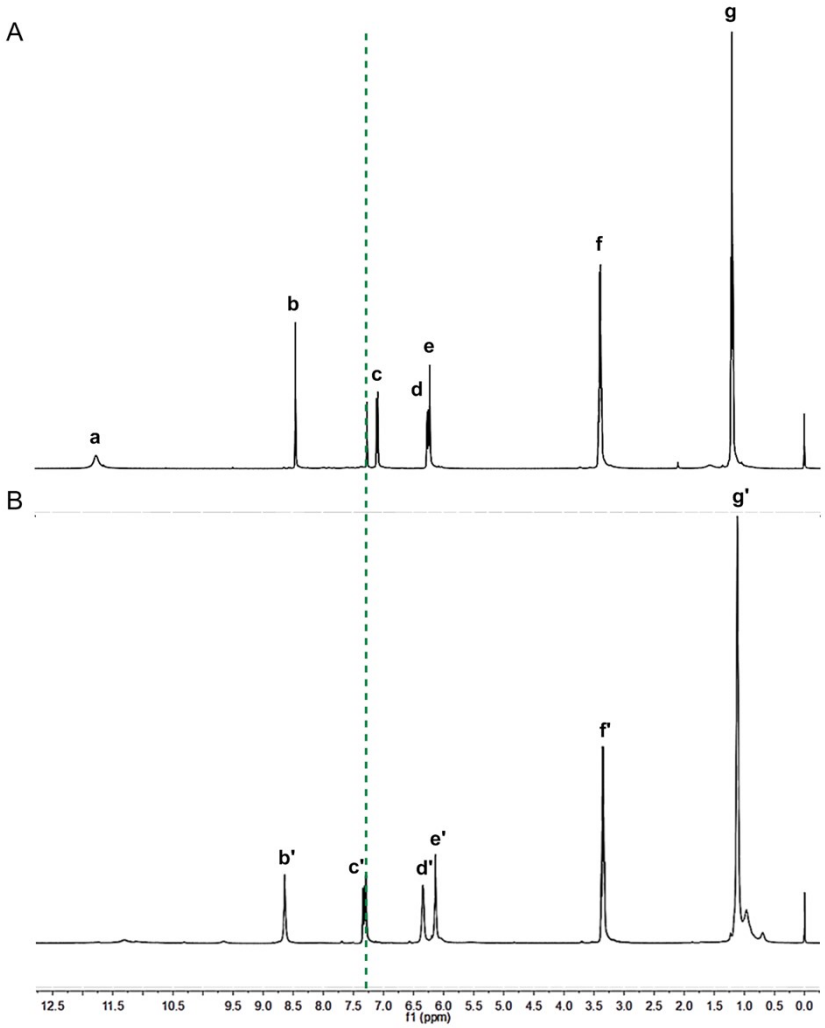

Fig. **S8** ^{1}H NMR of DHP and DHP–Cu^{2+} in $CDCl_3$ (A) only DHP, (B) DHP–Cu^{2+}

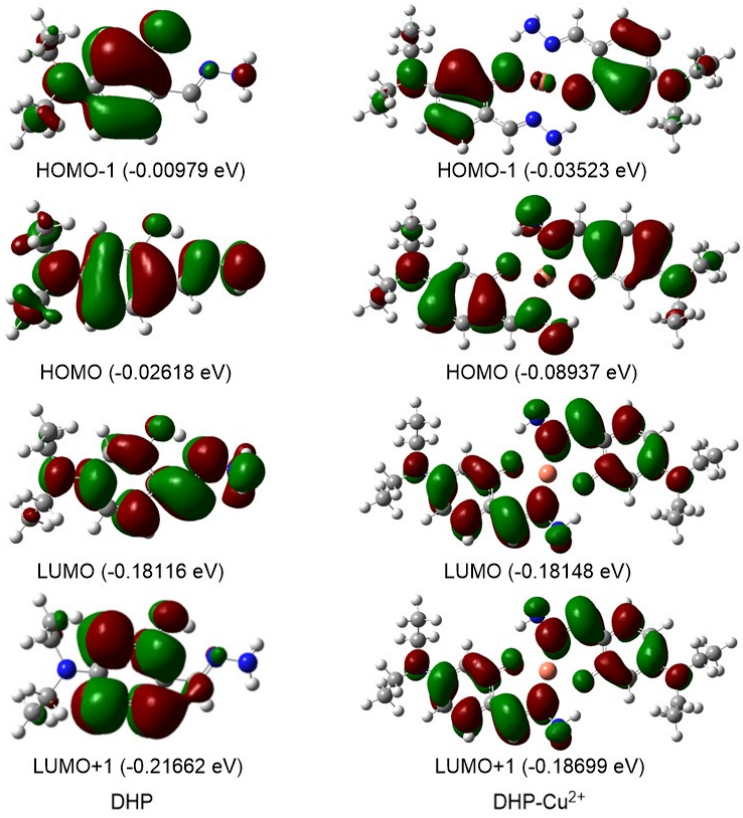

Fig. S9 Optimized molecular configuration and frontier orbitals of DHP and DHP–Cu^{2+}.

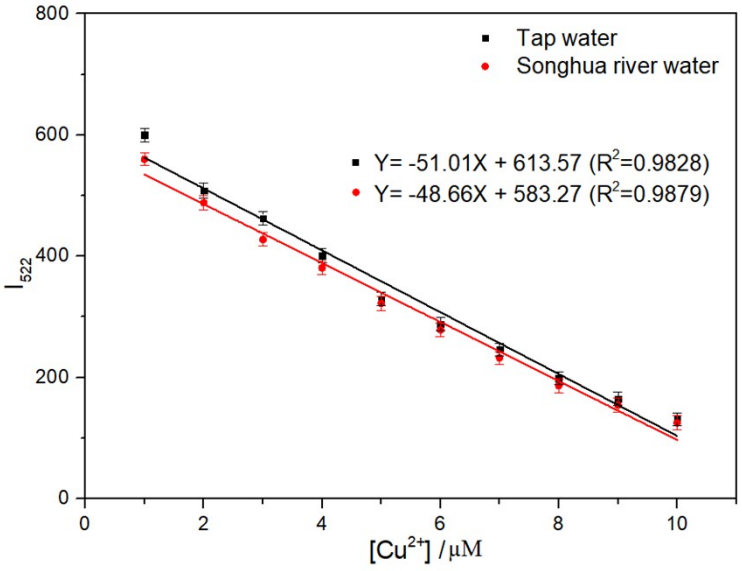

Fig. S10 Linear curves of fluorescence response of probe DHP to Cu^{2+} in tap water and river water.

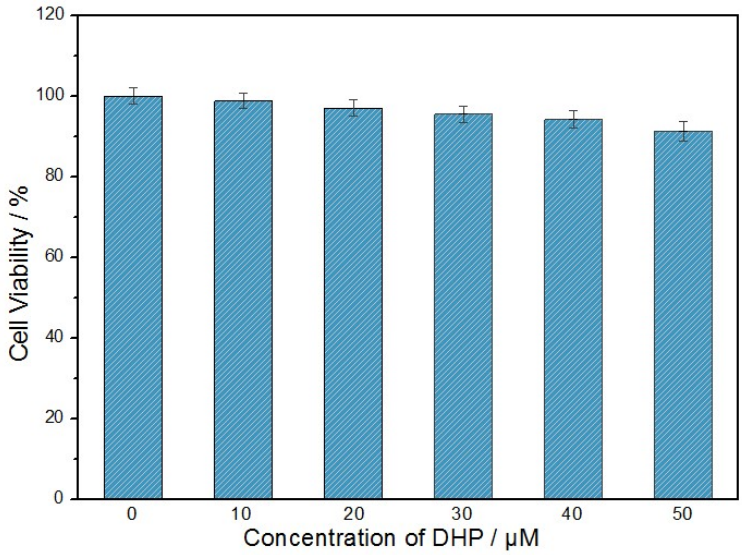

Fig. S11 Cell viability graph of probe DHP using HepG-2 cells by MTT assay after 24 h.

Table S1 Orbital energy differential of DHP and DHP–Cu^{2+}.

Compound	$\Delta E_{H\rightarrow L}$(A.U.)	$\Delta E_{H\rightarrow L+1}$(A.U.)	$\Delta E_{H-1\rightarrow L}$(A.U.)
DHP	0.15498	0.19044	0.17137
DHP–Cu^{2+}	0.09211	0.09762	0.14625

Reference

1 X. Tang, Z. Zhu, Y. Wang, J. Han, L. Ni, H. Q. Zhang, J. Li and Y. L. Mao, A cyanobiphenyl based fluorescent probe for rapid and specific detection of hypochlorite and its bio-imaging applications, Sens. Actuators B., 2018, **262**, 57–63.

2 J. Ahmad, R. Wahab, M. A. Siddiqui, N. N. Farshori, Q. Saquib, N. Ahmad and A. A. Al-Khedhairy, Neodymium oxide nanostructures and their cytotoxic evaluation in human cancer cells, J. Trace. Elem. Med. Bio., 2022, **73**, 127029.

3 L. Zhou, J. Cui, Z. Yu, D. Zou, W. Zhang and J. Qian, A β-d-galactose-guided fluorescent probe for selectively bioimaging endogenous formaldehyde in living HepG-2 cells, Sens. Actuators B., 2021, **332**, 129494.

4 Z. Li, J. Li, D. Zhang, X. Zhu, Y. Ye and Y. Zhao, A novel dual-channel fluorescent probe for nitroxyl detection and its application in HepG-2 cells, Sens. Actuators B., 2020, **312**, 127944.