Supporting Information

A turn-off fluorescent chemical sensor based on Thiazole-Schiff base structure for highly selective and accurate detection of Cu^{2+} in living cells

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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. Cells were inoculated overnight 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 HTT 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 cell viability in the HepG-2 cell line was calculated using the following equation:[2]

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

1.3 Cell fluorescence imaging

During cell imaging 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 HTT and different concentrations of Cu^{2+} ions (0, 20, 40, 60, 80, 100 μM) were added and incubated for 30 min, respectively. After each step, the cells in each well were washed three times with PBS buffer. The cells were eventually fixed on a circular slide and imaged by confocal electron fluorescence microscopy.[3,4]

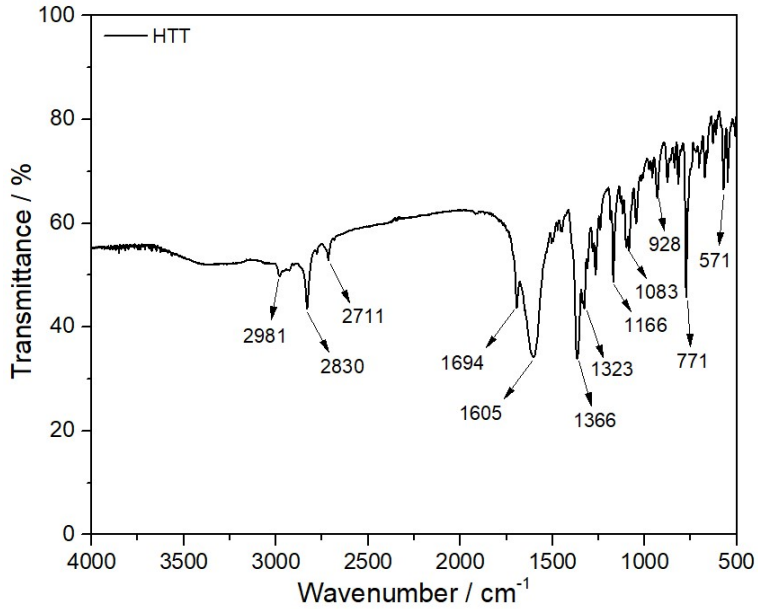


Fig. S1 IR spectrum of HTT

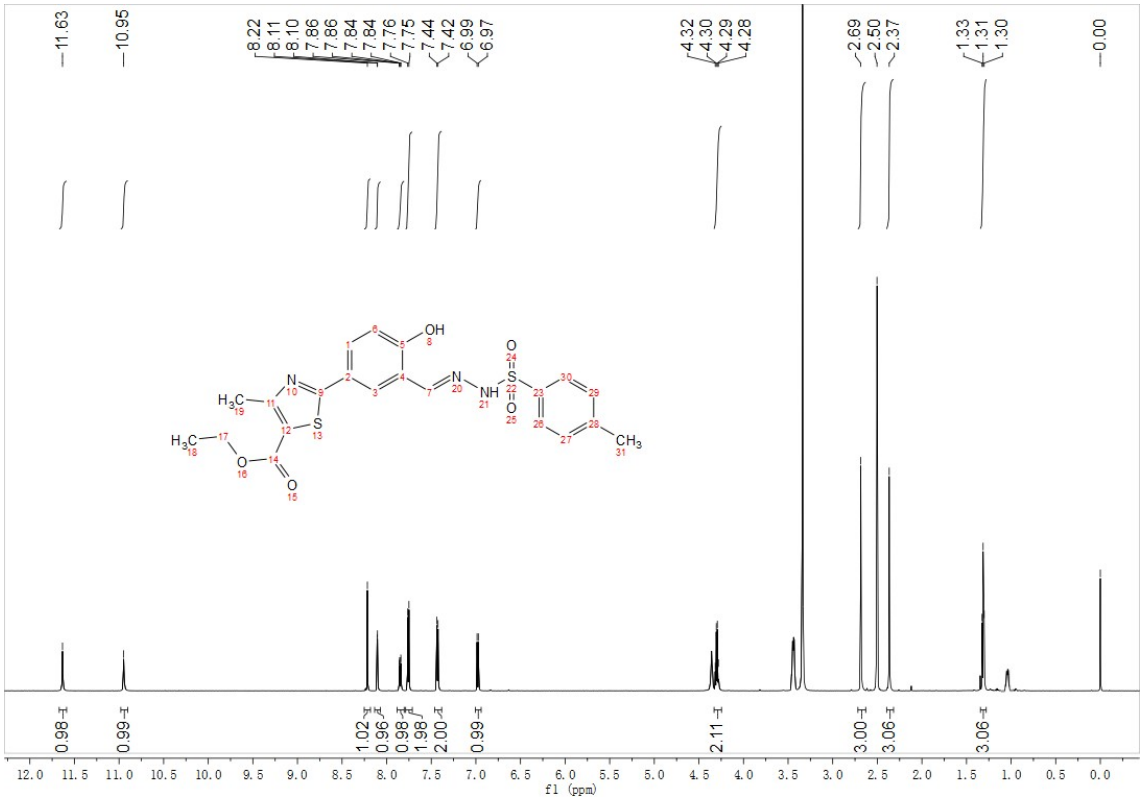


Fig. S2 ^{1}H NMR spectrum of HTT

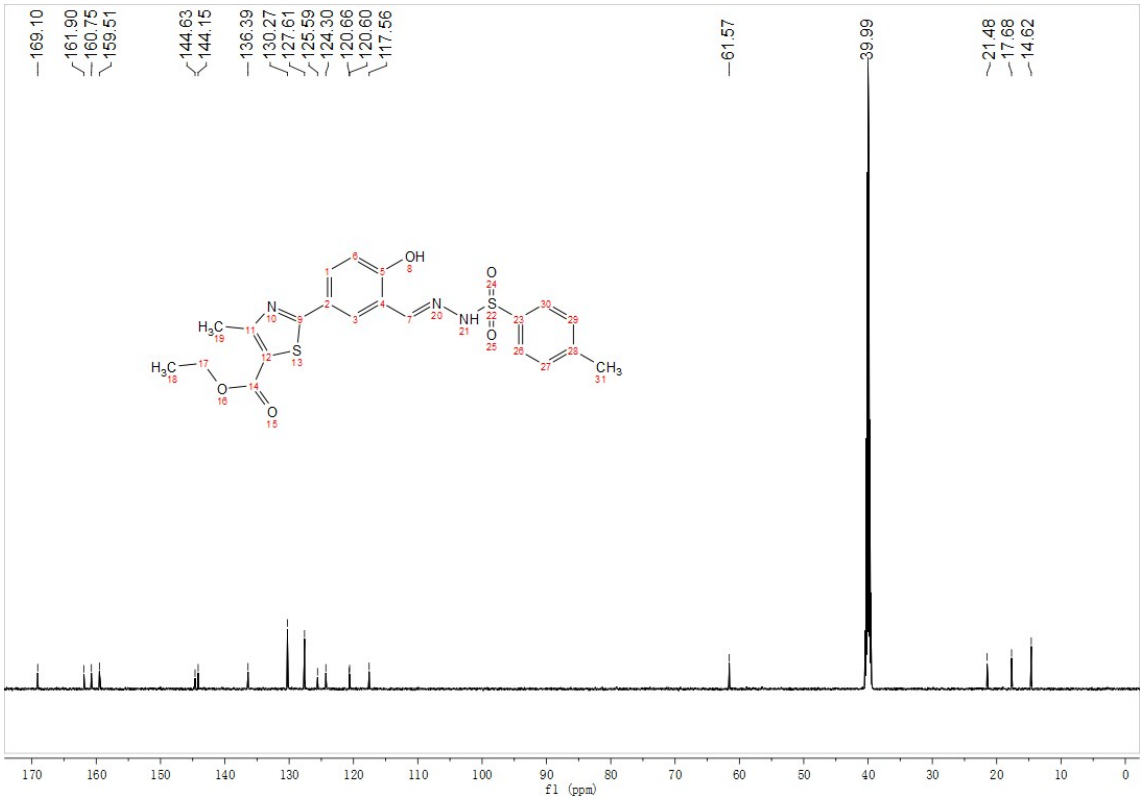


Fig. S3 ^{13}C NMR spectrum of HTT

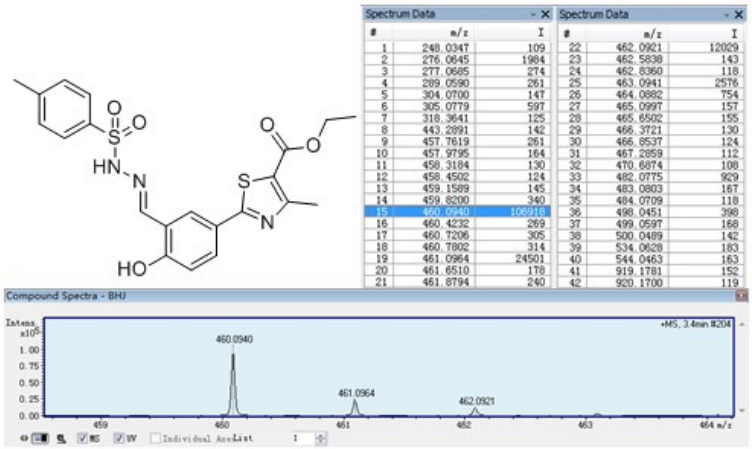


Fig. S4 HR-MS spectrum of HTT

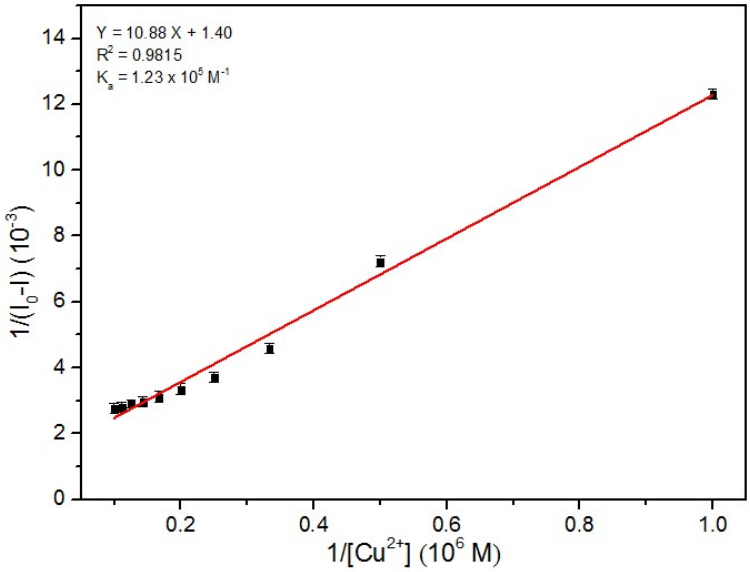


Fig. S5 Job's plot for determining the stoichiometry of HTT−Cu^{2+} in EtOH/H_2O (1/1, v/v).

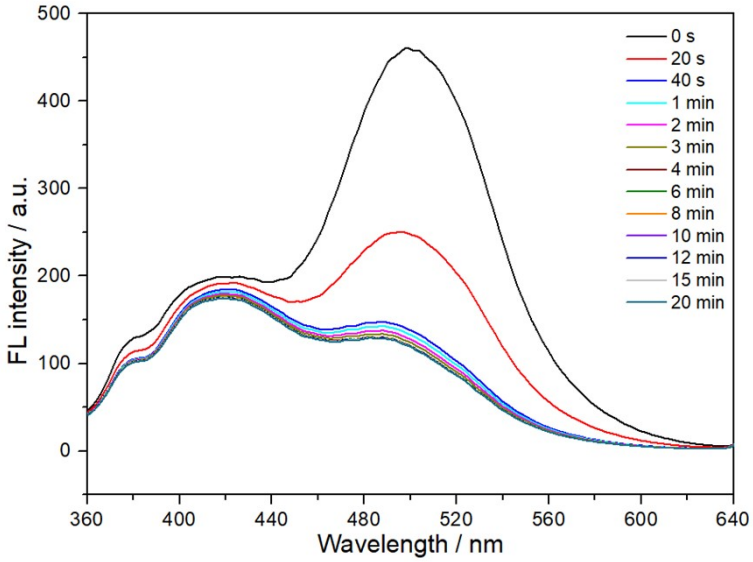


Fig. S6 Response time and stability of probe HTT (10 μM). Fluorescence intensity of HTT–Cu^{2+} (10 μM), EtOH/H_2O (1:1, v/v)

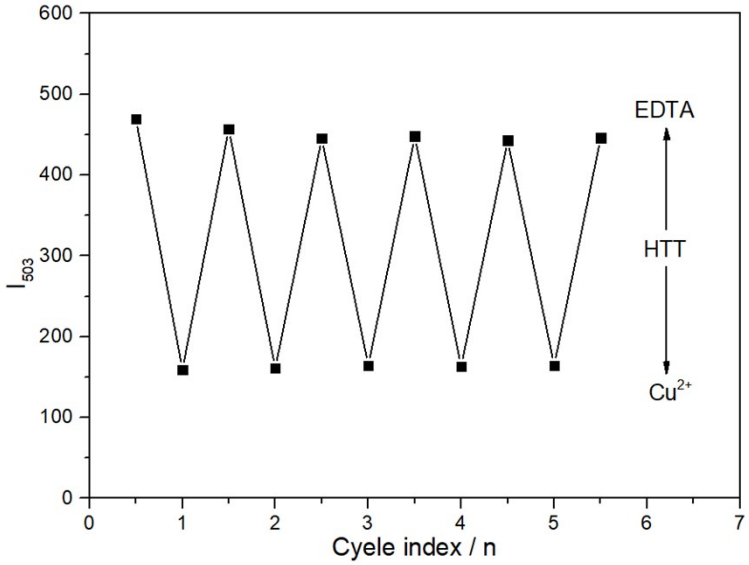


Fig. S7. The reversibility studies of HTT–Cu^{2+} with EDTA.

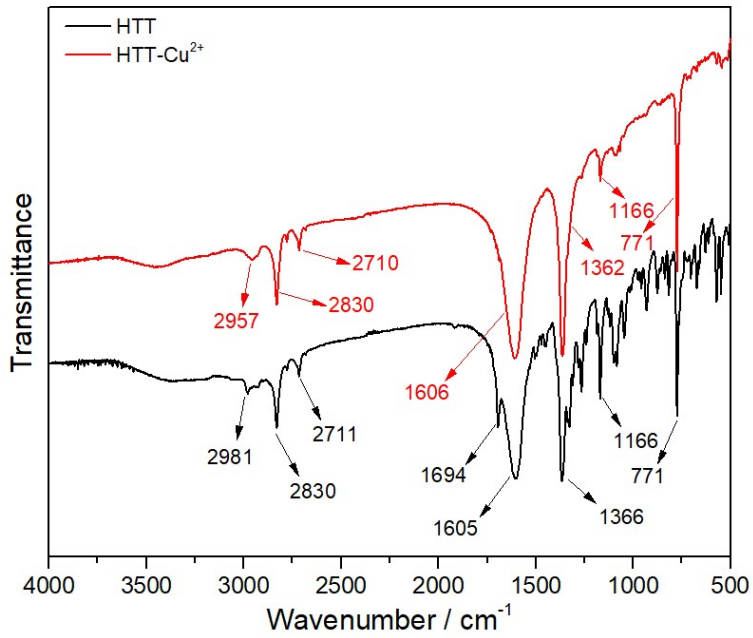


Fig. **S8** IR spectrum of HTT and HTT–Cu^{2+}

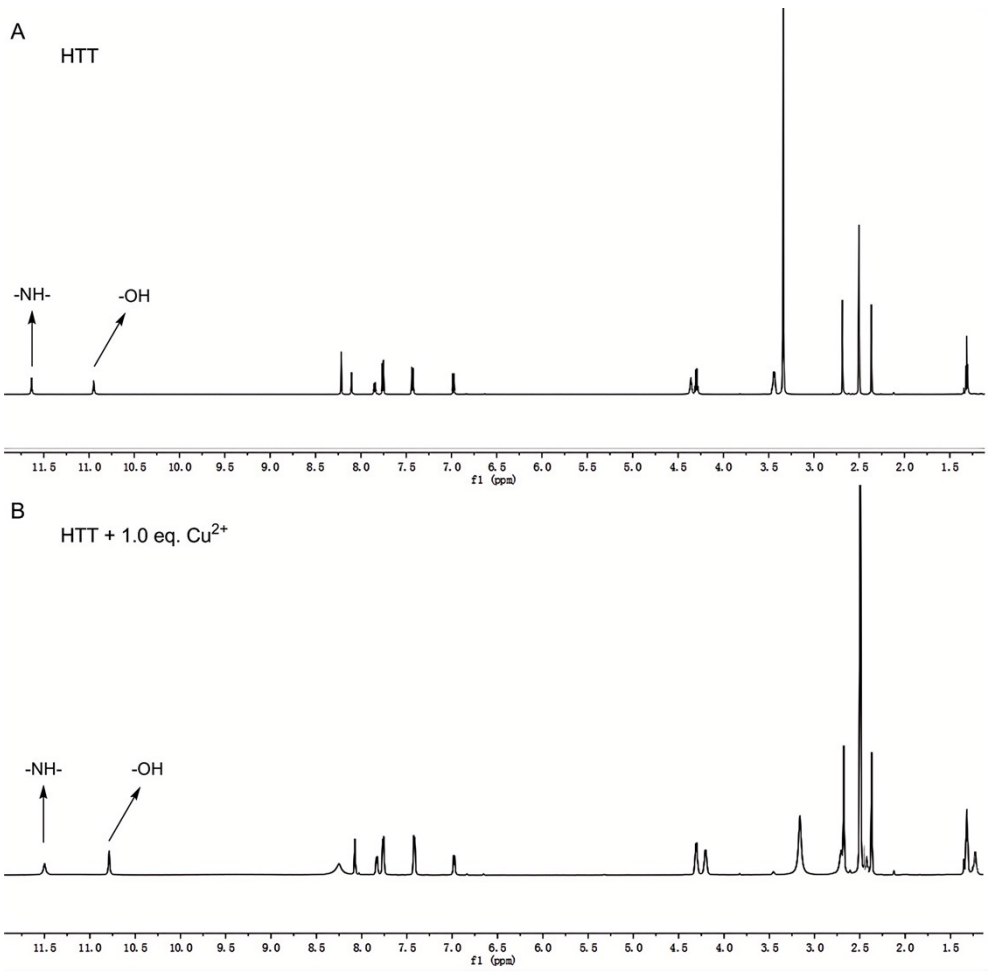


Fig. **S9** ^{1}H NMR of HTT and HTT–Cu^{2+} in DMSO (A) only HTT, (B) HTT–Cu^{2+}

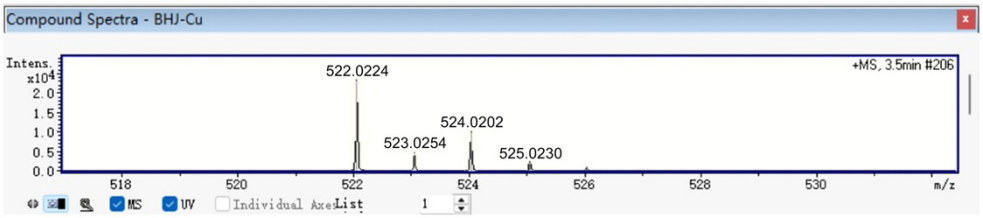


Fig. S10 HR-MS spectrum of HTT–Cu^{2+} complex

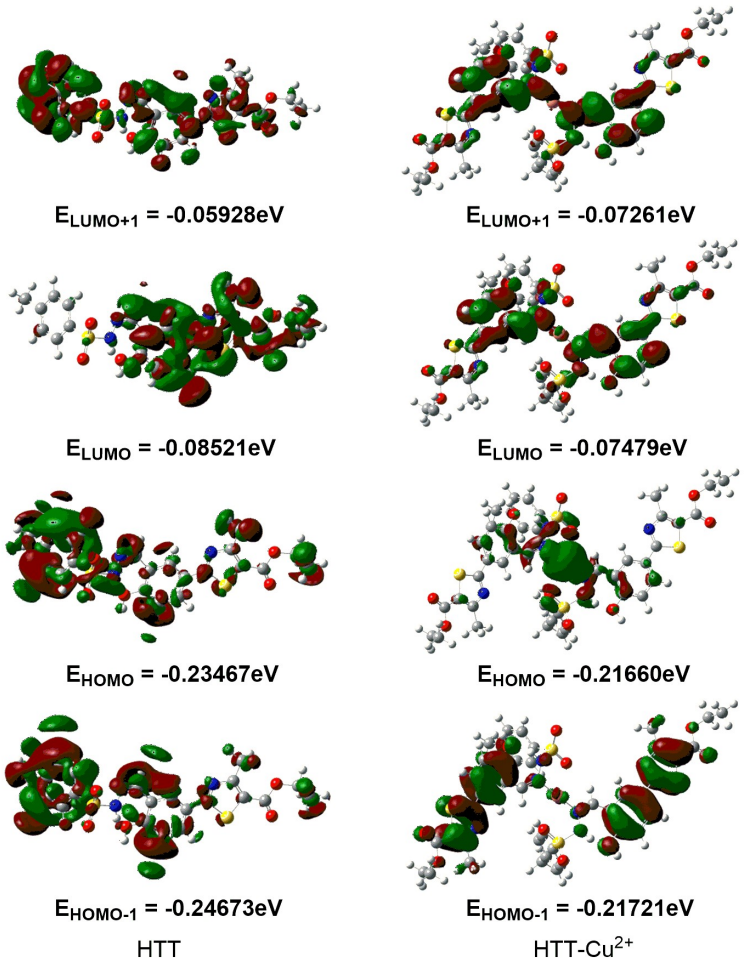


Fig. S11 Optimized molecular configuration and frontier orbitals of HTT and HTT–Cu^{2+}.

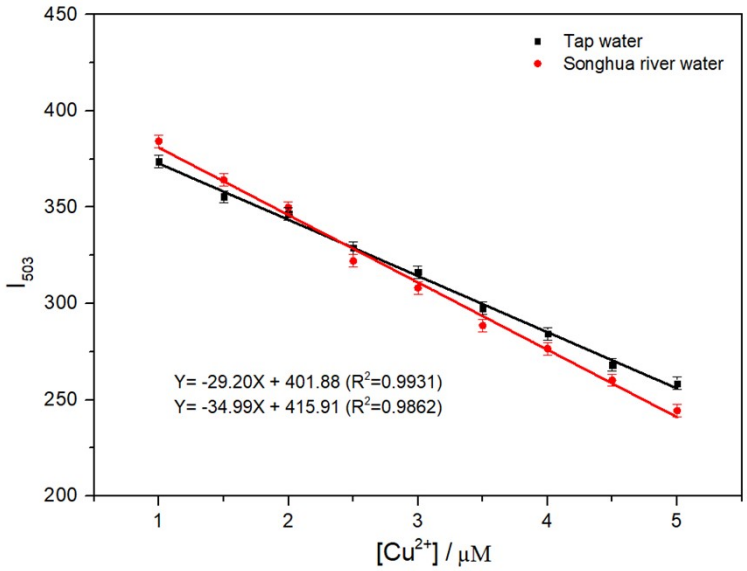


Fig. S12 Linear curves of fluorescence response of probe HTT to Cu^{2+} in tap water and river water.

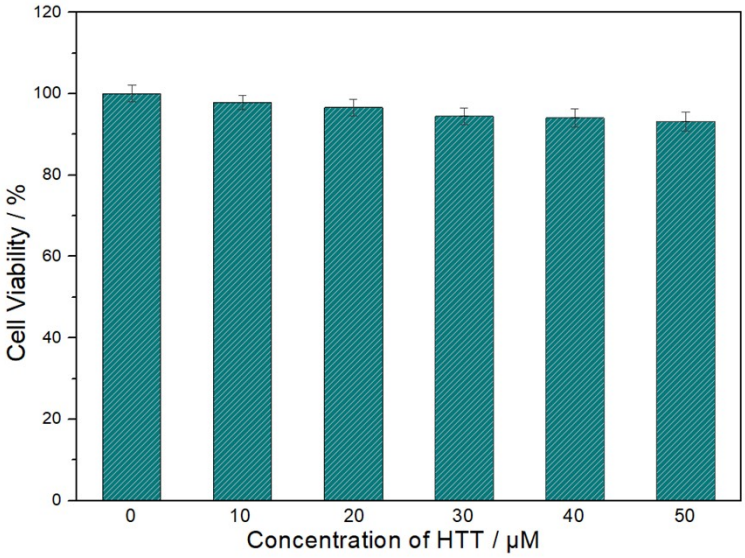
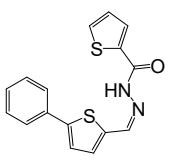


Fig. S13 Cell viability graph of probe HTT using HepG-2 cells by MTT assay after 24 h.

Table S1 Comparison of HTT with some Schiff base sensors for Cu^{2+} monitoring in previous literature

Fluorophor	Selectivity	Testing media	Practical application	LOD (M)	Reference
	Cu^{2+}	H_2O/CH_3CN (1:9)	Live cell imaging	2×10^{-8}	[5]

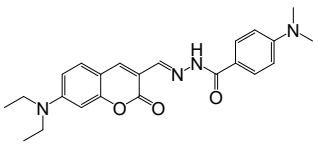
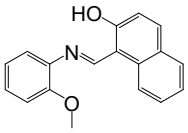
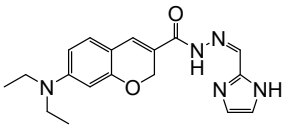
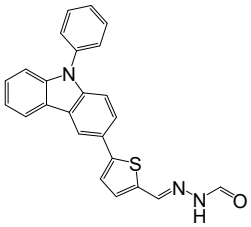
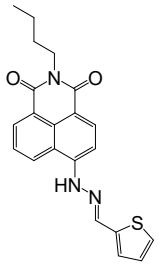
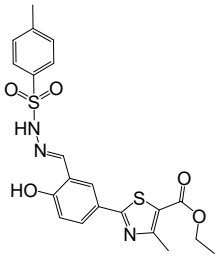
	Cu^{2+}	CH_3OH/H_2O (1:1)	Live cell imaging	2.4×10^{-8}	[6]
	Cu^{2+}	CH_3OH/H_2O (2:3)	Water sample	4.4×10^{-8}	[7]
	Cu^{2+}	CH_3CN/HEPES (3:2)	Live cell imaging	1.2×10^{-7}	[8]
	Cu^{2+}	$DMSO/H_2O$ (3:2)	Test strips	1.4×10^{-7}	[9]
	Cu^{2+}	CH_3CN/H_2O (3:1)	Live cell imaging	9.15×10^{-9}	[10]
	Cu^{2+}	$EtOH/H_2O$ (1:1)	Live cell imaging	1.1×10^{-9}	Current work

Table S2 Orbital energy differential of HTT and HTT–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.)
HTT	0.14946	0.17539	0.16152
HTT–Cu^{2+}	0.14181	0.14399	0.14242

Reference

1. X. Tang, Z. Zhu, Y. Wang, J. Han, L. Ni, H. Zhang, J. Li and M. Yanli, *Sensor. Actuat. B-Chem.*, 2018, **262**, 57-63.
2. J. Ahmad, R. Wahab, M. A. Siddiqui, N. N. Farshori, Q. Saquib, N. Ahmad and A. A. Al-Khedhairy, *J. Trace Elem. Med. Bio.*, 2022, **73**, 127029.
3. L. Zhou, J. Cui, Z. Yu, D. Zou, W. Zhang and J. Qian, *Sensor. Actuat. B-Chem.*, 2021, **332**, 129494.
4. Z. Li, J. Li, D. Zhang, X. Zhu, Y. Ye and Y. Zhao, *Sensor. Actuat. B-Chem.*, 2020, **312**, 127944.
5. P. Dhanapal and M. S.L, *Synthetic Met.*, 2024, **309**, 117752.
6. Z.-G. Wang, X.-J. Ding, Y.-Y. Huang, X.-J. Yan, B. Ding, Q.-Z. Li, C.-Z. Xie and J.-Y. Xu, *Dyes Pigm.*, 2020, **175**, 108156.
7. M. A. M. Alhamami, A. Y. A. Mohammed, J. S. Algethami, H. M. Al-Saidi, S. Khan and S. S. Alharthi, *Microchem. J.*, 2024, **197**, 109817.
8. G. He, X. Hua, N. Yang, L. Li, J. Xu, L. Yang, Q. Wang and L. Ji, *Bioorg. Chem.*, 2019, **91**, 103176.
9. L. Wang, Y. Chen, Z. Xing, L. Wang and J. Ma, *J. Mol. Struct.*, 2025, **1321**, 140268.
10. Y.-L. Liu, L. Yang, P. Li, S.-J. Li, L. Li, X.-X. Pang, F. Ye and Y. Fu, *Spectrochim. Acta A*, 2020, **227**, 117540.