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Supporting Information

Mitochondria-targeted fluorescent probe with pH/viscosity response for assisted detection of non-alcoholic fatty liver in mice

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S1. Experimental section

1. X-ray Crystallography

Crystallographic data for **HTC** were collected on a Bruker APEX 2 CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) in the ω-scan mode. [1] The structure was solved by a charge flipping algorithm and refined by full-matrix least-squares methods on F^2. [2,3] The structural data reported in this

paper have been deposited at the Cambridge Crystallographic Data Centre, which contains supplementary crystallographic data to this paper. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

2. Probe Viscosity Fluorescence Detection

In this study, a mixture of glycerol (99% pure) and distilled water was selected as the testing system. Initially, a stock solution of the probe was prepared at a concentration of 1×10^{-3} mol·L^{-1}. Subsequently, glycerol-water solutions with varying proportions of glycerol, ranging from 0% to 90%, were prepared to obtain mixtures with different viscosities. After adding a specific concentration of the probe, the solution was subjected to ultrasound treatment for 10 min. to ensure thorough mixing. The mixture was then allowed to stand for 1 h before fluorescence spectra were measured using a fluorescence spectrophotometer.

3. Cell Incubation and Imaging

Cell Culture System for Normal and Cancer Cells. The cell culture system consisted of 1% antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin), 10% fetal bovine serum (FBS), and either DMEM or RPMI 1640 medium. After digesting and centrifuging the cells, the cell suspension (approximately 6×10^4 cells/mL) was added to a confocal culture dish (1 mL) and placed in an incubator for 24 h until the cell density reached approximately 70%–90%. The cells were then washed twice with pre-warmed phosphate-buffered saline (PBS).

4. *In Vivo* Imaging of Probes in Mice

All animals used in this study were purchased from the Experimental Animal Center of Guizhou Medical University (Production License: SCXK (Xiang) 2022-0011). C57BL/6 mice (6 weeks old, male) were obtained from Changsha Tianqin Biotechnology Co., Ltd. The experiments were conducted in accordance with the

National Institutes of Health guidelines for the care and use of laboratory animals. Mice were divided into a control group and a model group. The model group was fed a high-fat diet and received intraperitoneal injections of olive oil containing carbon tetrachloride (CCl_4: olive oil = 1:4) for a duration of two weeks. Prior to the experiment, all mice were fasted for 12 h. The experimental and control groups received intraperitoneal injections of HTC (20 μM, 100 μL). Following this, the mice were anesthetized, and *in vivo* optical imaging was performed using the IVIS Lumina Series III (Excitation: 520 nm, Emission: 630 nm, Exposure time: 8 s) to observe the mice. Finally, the mice were euthanized, and key organs were dissected for imaging.

The synthetic procedure of HTC is shown in the figure S1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.68 (d, J = 15.7 Hz, 1H), 8.20 (d, J = 4.1 Hz, 1H), 7.92 – 7.84 (m, 2H), 7.70 (dd, J = 6.4, 2.3 Hz, 3H), 7.64 – 7.55 (m, 2H), 7.19 (d, J = 15.7 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 4.62 (d, J = 7.3 Hz, 2H), 1.78 (s, 6H), 1.43 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.86, 159.52, 155.35, 146.24, 143.49, 140.54, 140.41, 137.89, 129.06, 128.74, 128.00, 124.93, 123.56, 123.04, 116.34, 114.48, 108.64, 51.72, 41.51, 39.52, 25.79, 13.51ppm. ESI-TOF: $[M]^+$ calcd for $C_{24}H_{24}NOS^+$: 374.1573; found: 374.15654.

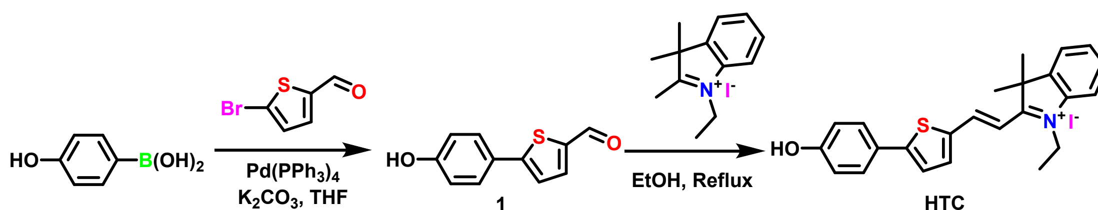


Figure S1. The synthesis route of **HTC**.

HRMS spectra

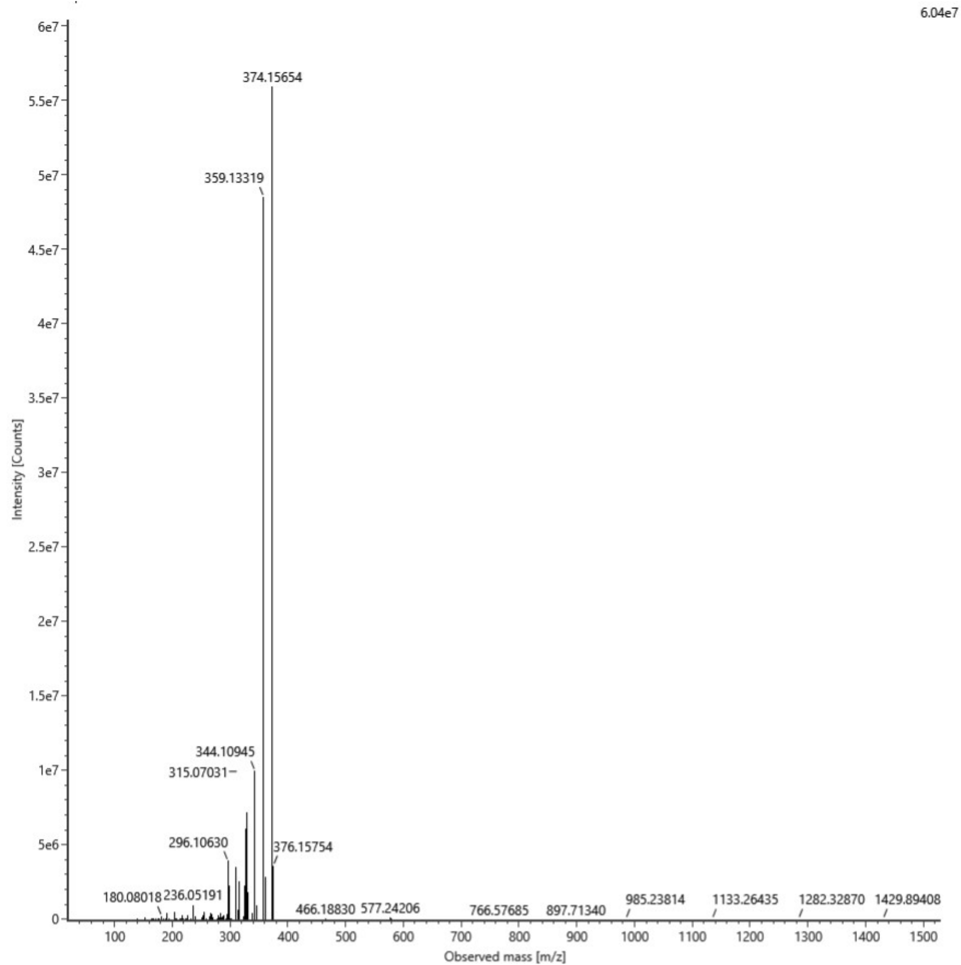


Figure S2. High-Resolution Mass Spectrum of HTC.

NMR spectra

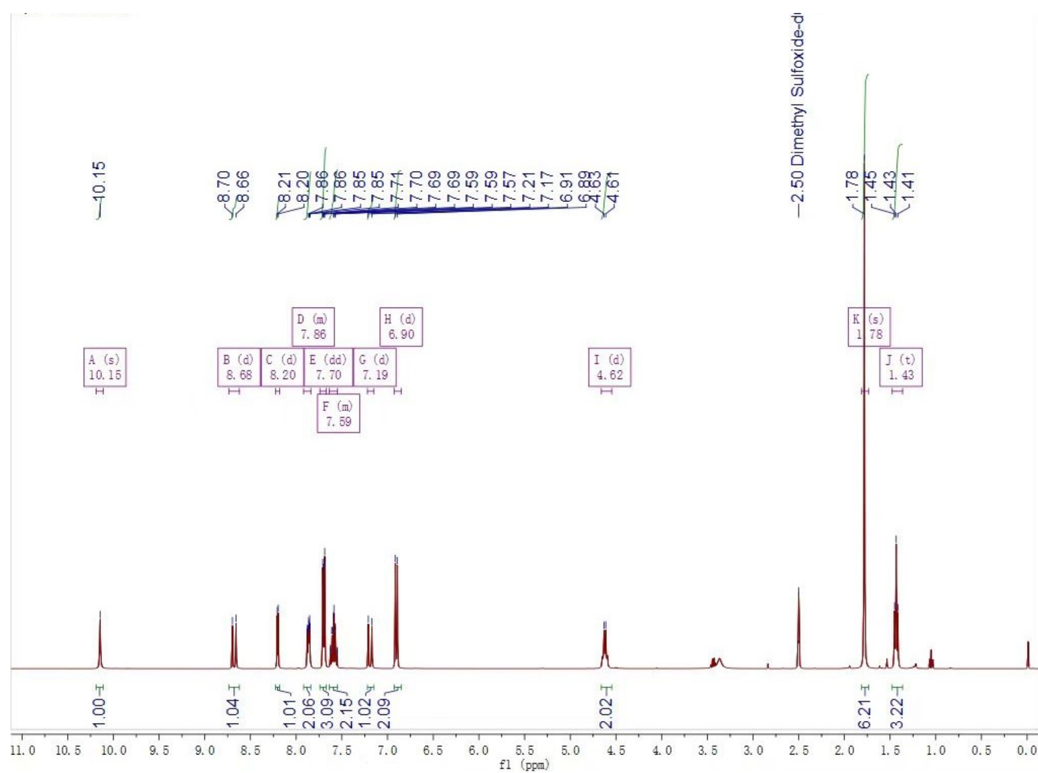


Figure S3. ^{1}H NMR spectrum of **HTC**.

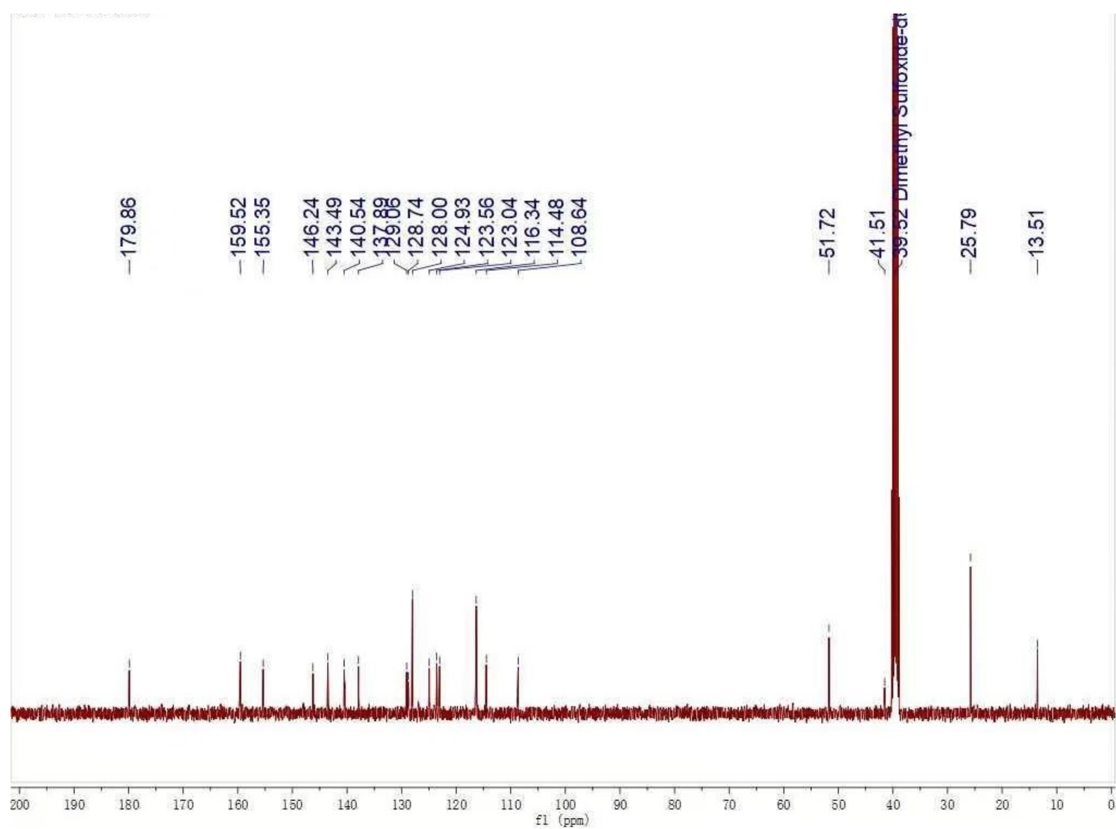


Figure S4. ^{13}C NMR spectrum of **HTC**.

Single crystal X-ray diffraction analysis

Table S1. Summary of crystal data for **HTC**.

Parameter	**HTC**
CCDC	2385840
Empirical formula	$C_{24}H_{24}INOS$
Formula weight [g mol^{-1}]	501.40
Crystal system	monoclinic
Space group	$P2_1/c$
a [Å]	20.9571(15)
b [Å]	7.5874(5)
c [Å]	14.0261(10)
α	90
β	98.088(3)
γ	90
Volume [Å^3]	2208.1(3)
Z	4
Density, calcd [gm^{-3}]	1.508
Temperature [K]	273(2)
Unique reflns	2516
Obsd reflns	4022
Parameters	257
R_{int}	0.0960
$R[I>2\sigma(I)]^a$	0.0829
W[all data]R^b	0.1198
GOF on F^2	1.120

Table S2. Hydrogen-bond geometry (Å, °).

D—H···A	D—H(Å)	H···A(Å)	D···A(Å)	D—H···A(°)
O1—H1···I1	0.82	2.66	3.451 (6)	162.7
C12—H12···O1^i	0.93	2.50	3.387 (10)	159.4
C23—H23A···O1^i	0.97	2.30	3.222 (10)	158.4

Symmetry codes: ^{i}−x+1, y−1/2, −z+1/2

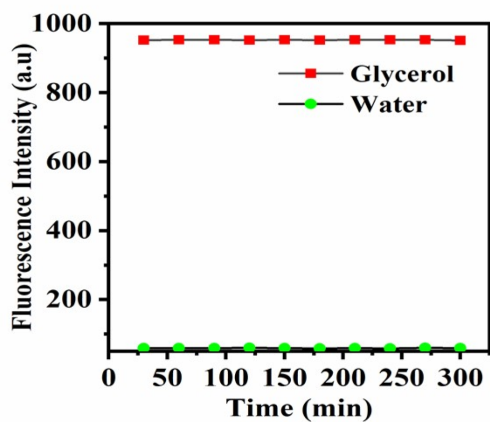


Figure S5. Fluorescence Intensity of **HTC** (10 μM) over Time in Water and Glycerol.

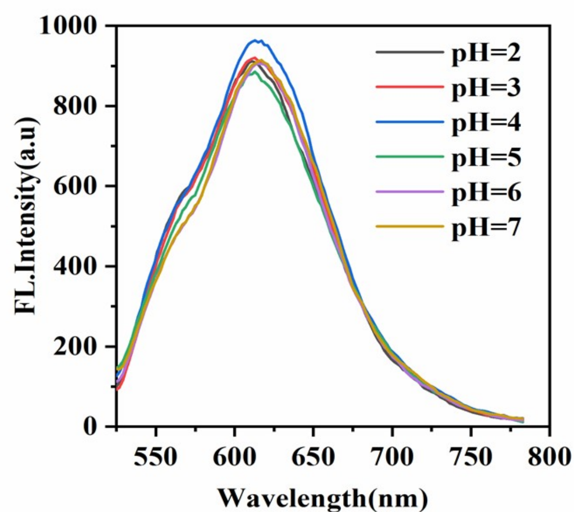


Figure S6. Fluorescence Spectrum of Probe **HTC** at pH 2 to 7.

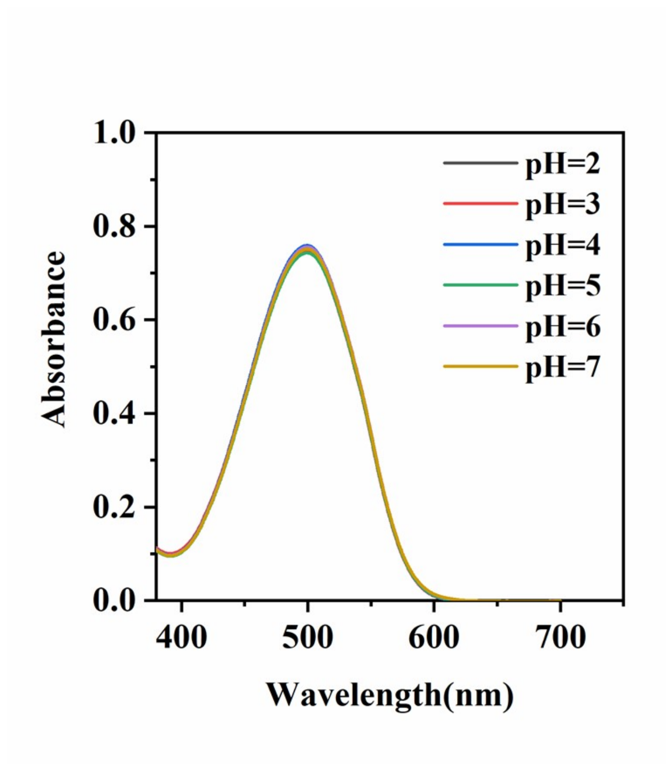
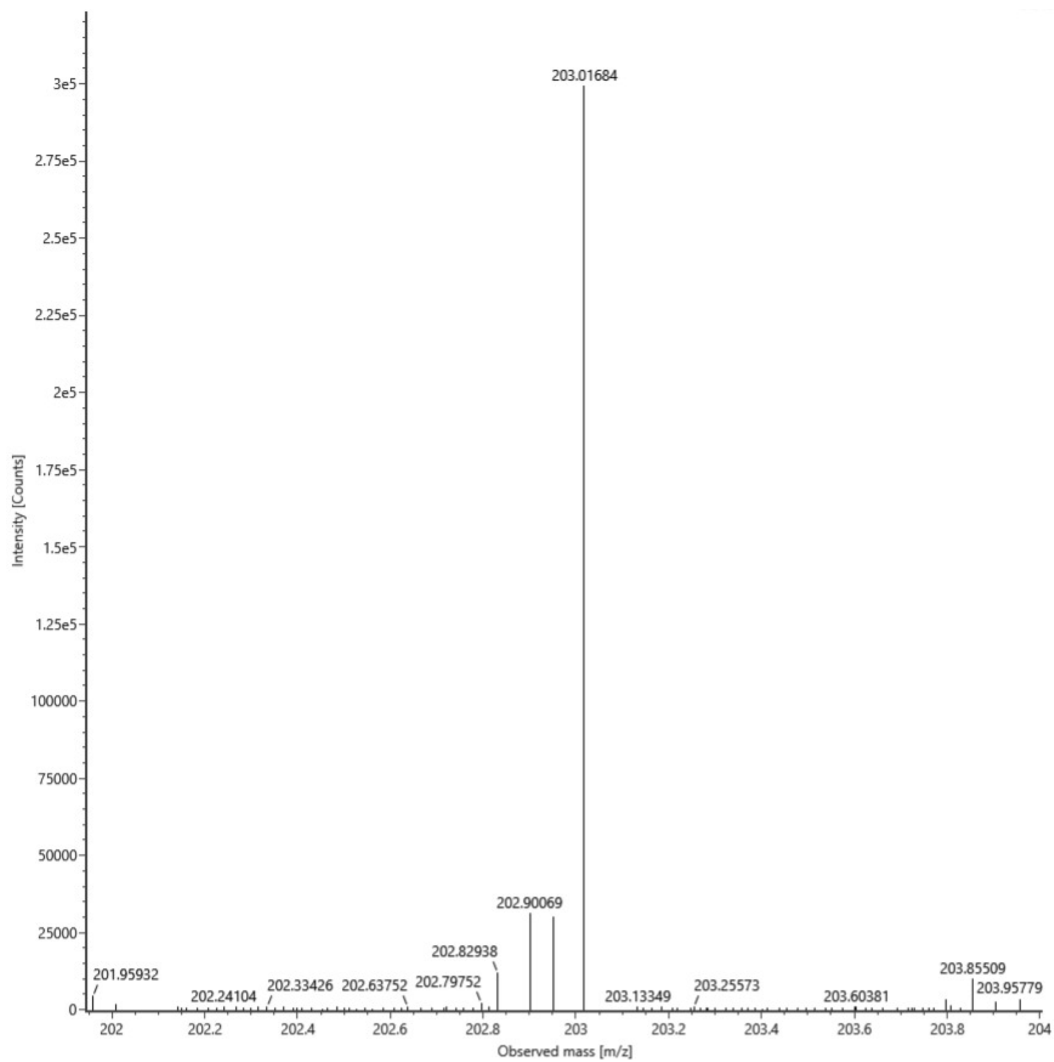


Figure S7. UV-Vis Absorption Spectrum of Probe **HTC** at pH 2 to 7.

(a)



(b)

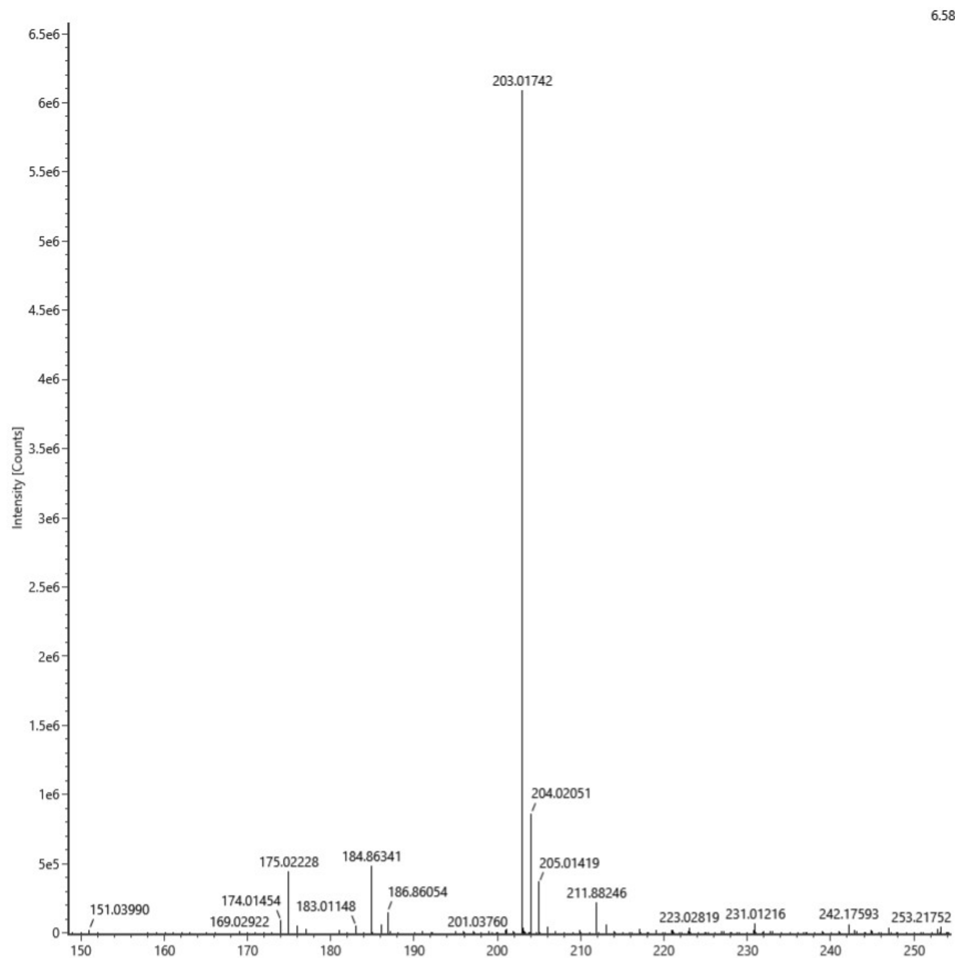


Figure S8. Mass Spectra at (a) pH 11 and (b) 12.

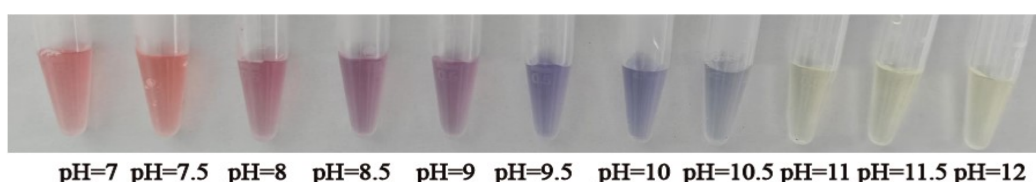


Figure S9. Colour Change Spectra of Probe **HTC** Solution at pH 7 to 12.

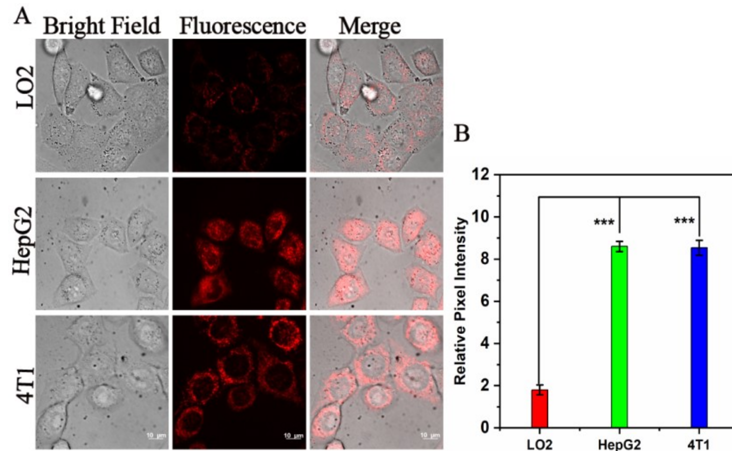


Figure S10. (A) Fluorescence Imaging of LO2, HepG2, and 4T1 Cells

Cells were incubated with **HTC** (5 μM) for 2 h.

(B) Relative Fluorescence Pixel Intensity. data are presented as mean ± SD (standard deviation, n =3), ***$p < 0.001$.

λ_{ex} = 520 nm, λ_{em} = 610 − 630 nm; scale bar: 10 μM.

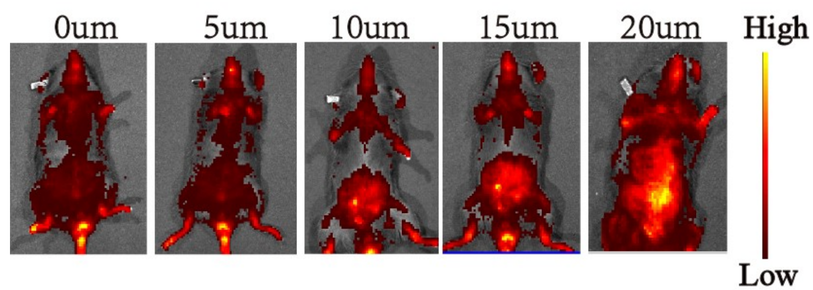


Figure S11. Imaging map of intraperitoneal injection of different concentrations of **HTC** in NAFLD mice.

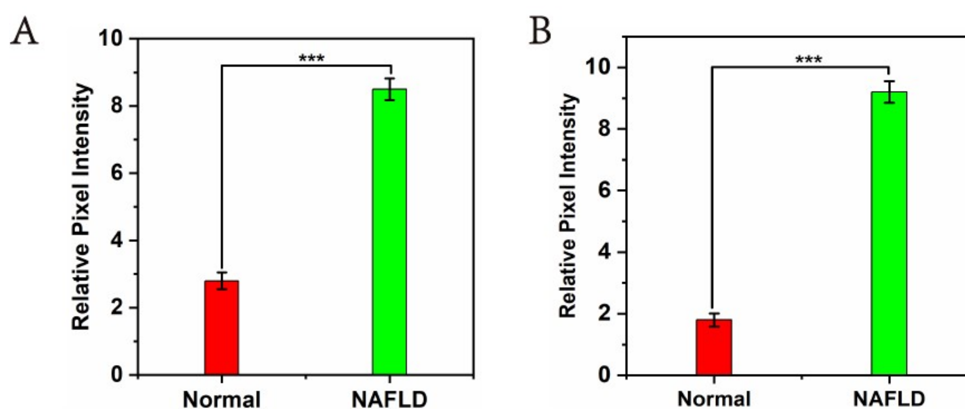


Figure S12. (A) Quantification of relative fluorescence intensity in the livers of normal and fatty liver mice; (B) Relative fluorescence intensity plot of metabolized urine from normal and fatty liver mice. λ_{ex}=520nm, λ_{em}=610-630 nm. data are presented as mean ± SD (standard deviation, *n* =3), ****p* < 0.001.

References

[1] *36SAINT* and *APEX 2* software for CCD diffractometers; Bruker AXS Inc.: Madison, USA, **2015**.

[2] G.M. Sheldrick, SHELXT - Integrated space-group and crystal structure determination, *Acta Crystallogr., Sect. A: Found. Adv.,* 2015, **71**, 3-8.

[3] A.L. Spek, PLATON SQUEEZE: a tool for the calculation of the disordered solvent contribution to the calculated structure factors, *Acta Crystallogr., Sect. C: Struct. Chem.,* 2015, **71**, 9-18.