Supplementary Information

Isocamphanyl-based fluorescent "turn-on" probe for highly sensitive and selective detection of Ga^{3+} and application in vivo and in vitro

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Content

1. ^{1}H-NMR, ^{13}C-NMR, IR and Mass spectra of HT

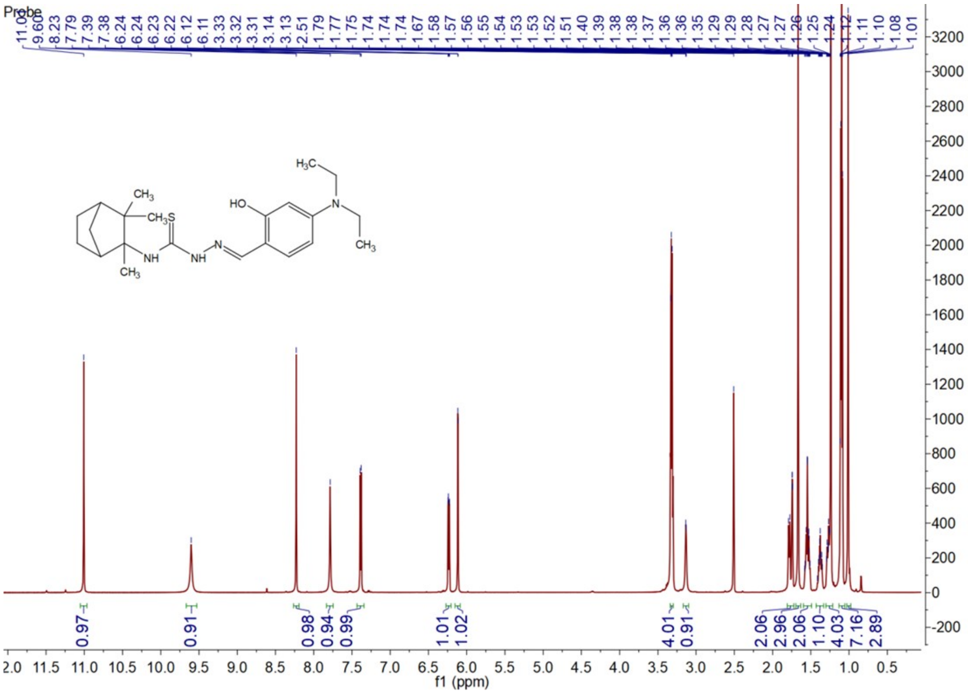


Fig. S1 ^{1}H-NMR Spectrum of HT in DMSO-d_6.

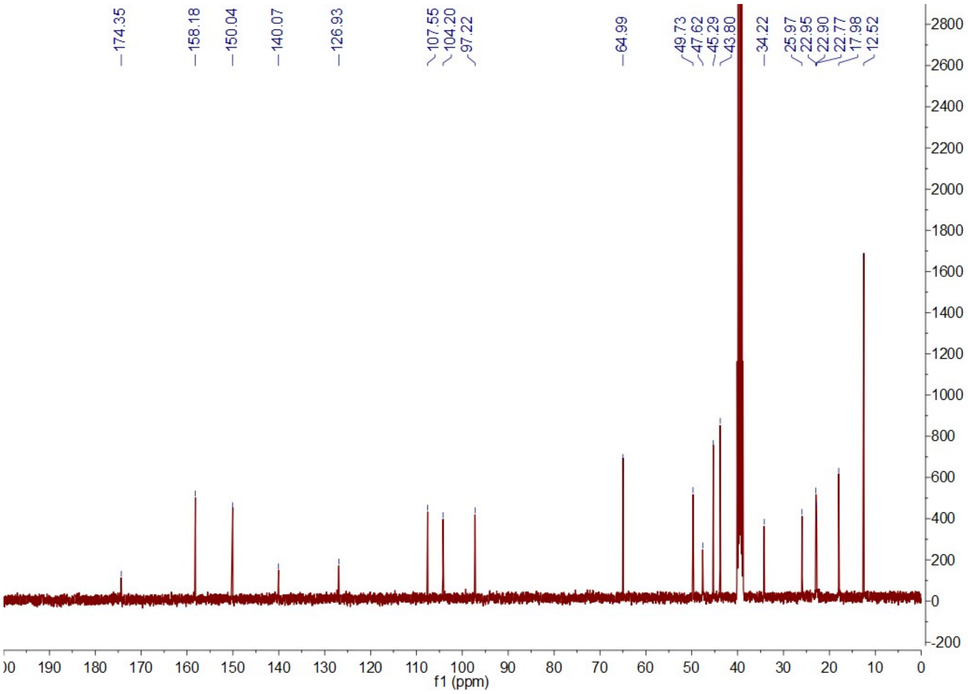


Fig. S2 ^{13}C-NMR Spectrum of HT in DMSO-d_6.

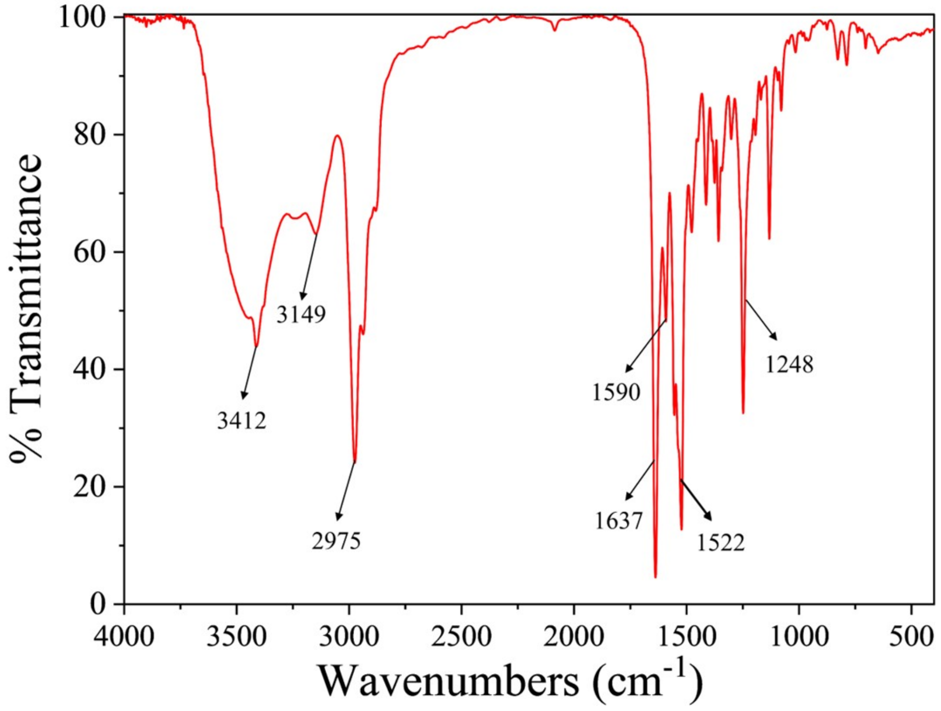


Fig. S3 IR Spectrum of HT.

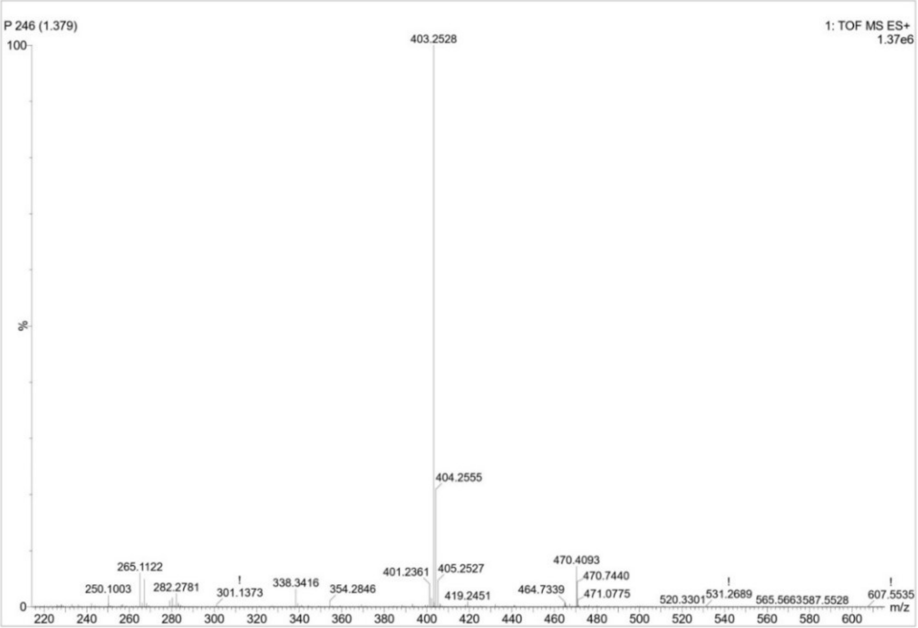


Fig. S4 Mass Spectrum of HT in CH_3OH.

2. The fluorescence spectra changes of HT in different solvents

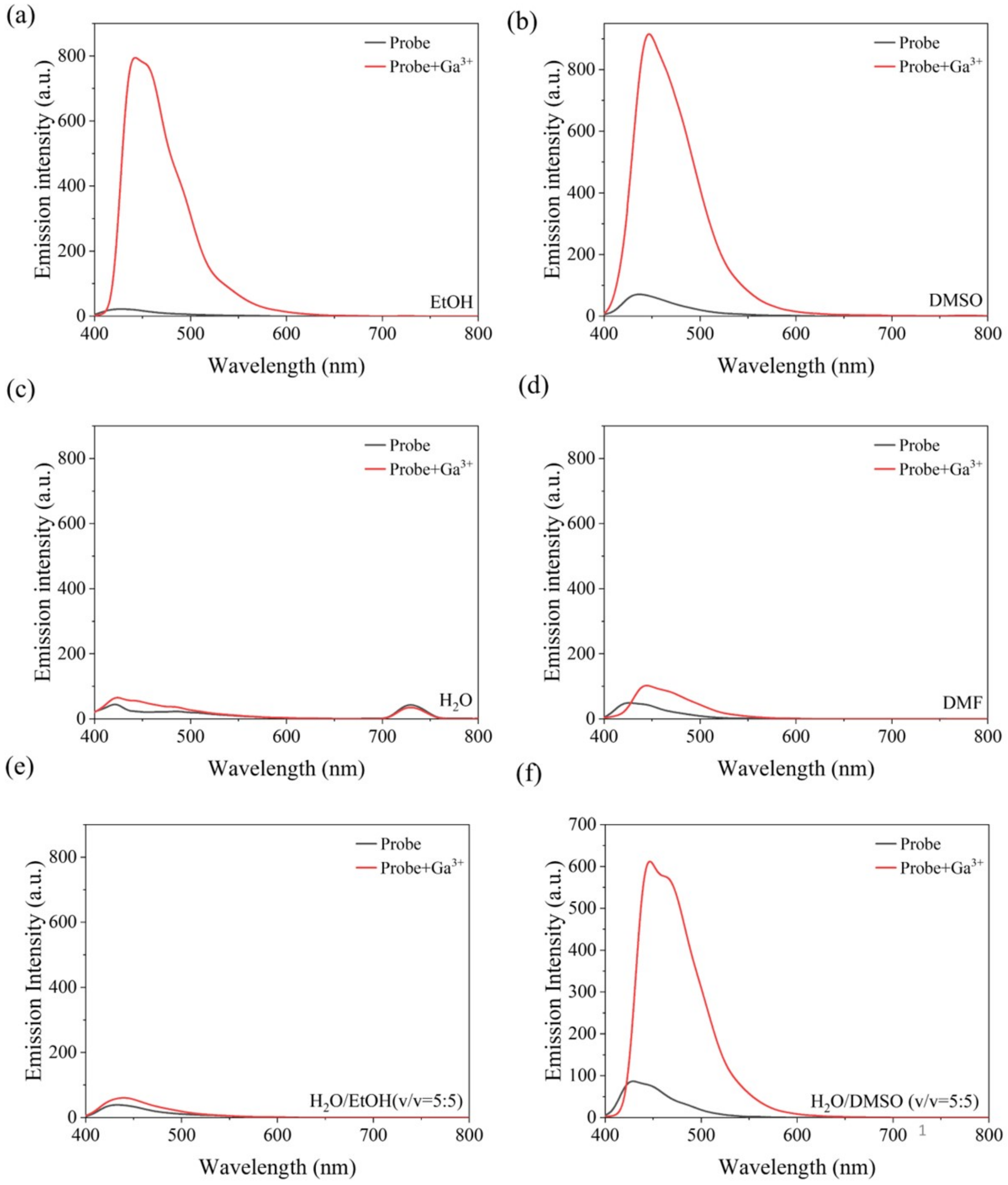


Fig. S5 The fluorescence spectra changes of HT with and without Ga^{3+} in different solvents.

3. The change of fluorescence intensity of HT towards Ga^{3+} in DMSO-water solution

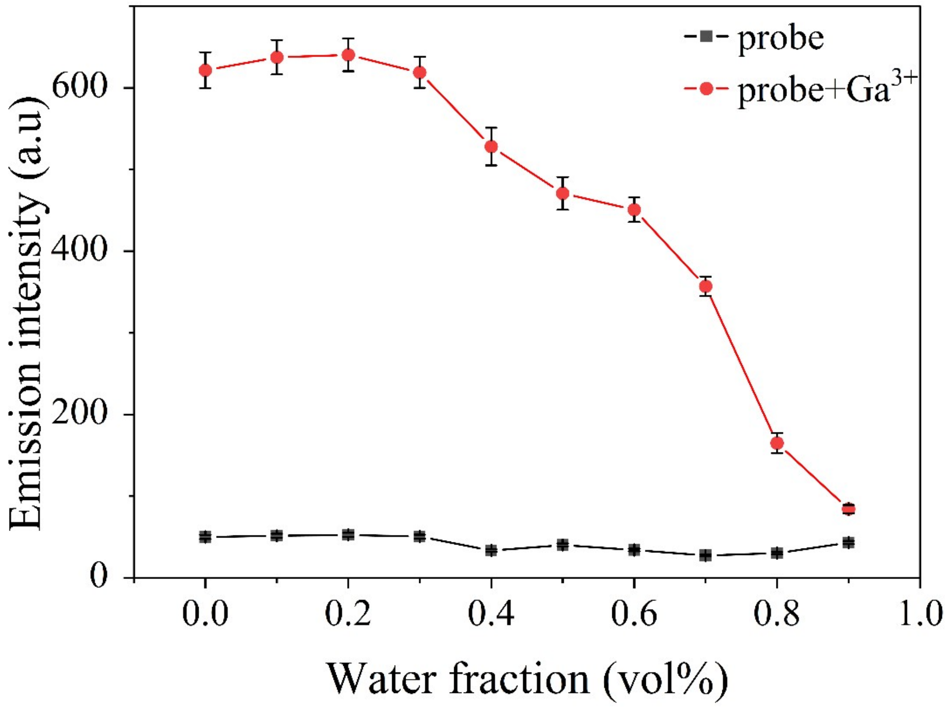


Fig. S6 The change of fluorescence intensity of HT (10 μM) at 447 nm upon the addition of Ga^{3+} ions (10 equiv.) in the solution of different amounts of DMSO and H_2O.

4. UV-Vis spectral change of HT with different metal ions

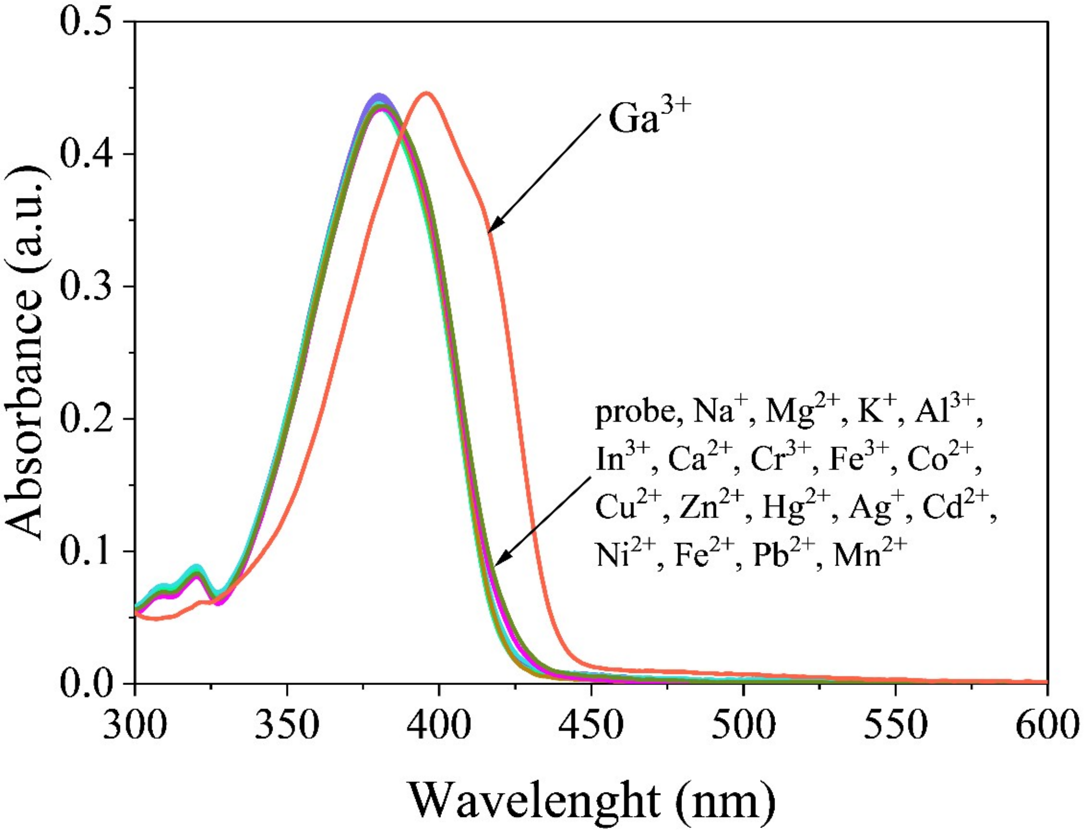


Fig. S7 The absorption spectra of HT (10 μM) with various metal ions (100 μM) in the H_2O/DMSO (v/v = 3/7).

5. The fluorescent intensity changes of HT-other metal ions upon the addition of Ga^{3+}

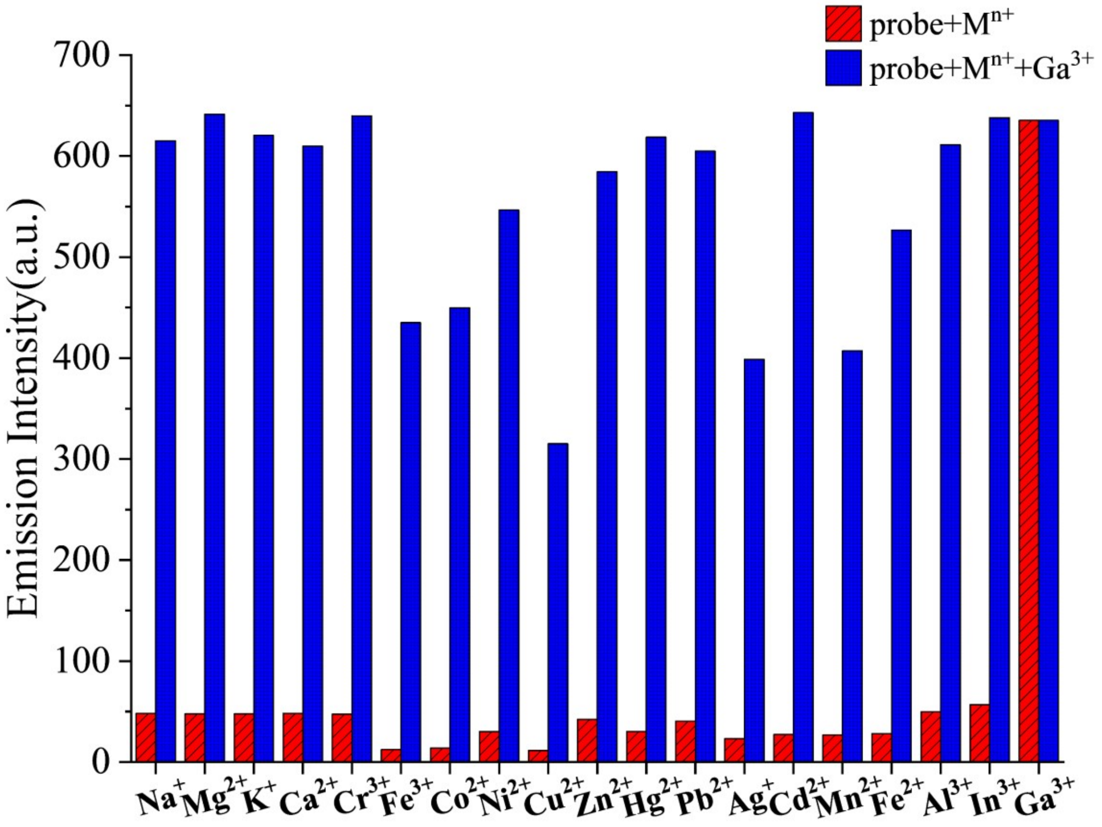


Fig. S8 The fluorescent intensity changes of HT-other metal ions (10 µM) upon the addition of Ga^{3+}(10 equiv.), red bars represent different ions, blue bars represent the subsequent addition of Ga^{3+} to aforementioned solutions.

6. Determining the binding stoichiometry and binding constant between HT and Ga^{3+}

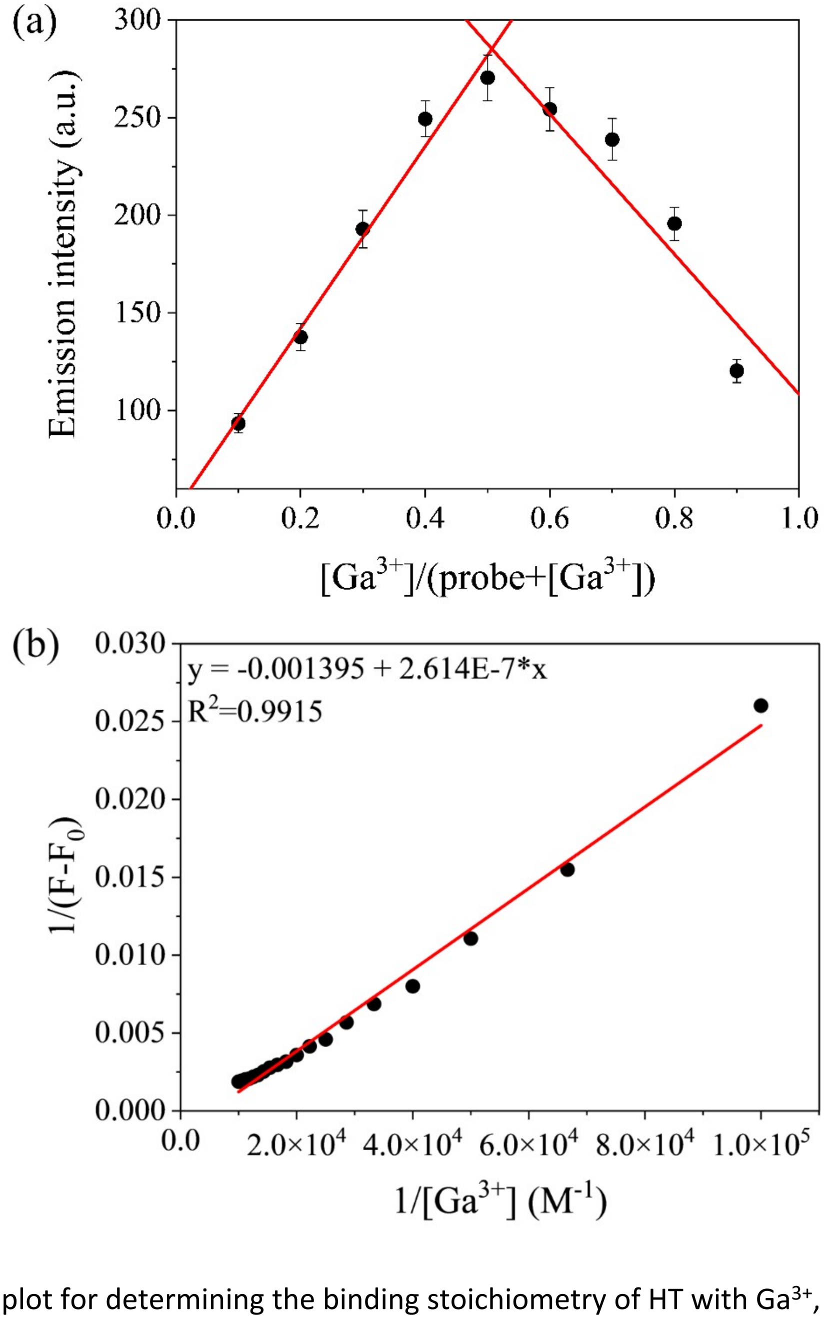


Fig. S9 (a) Job's plot for determining the binding stoichiometry of HT with Ga^{3+}, where the total concentration of HT and Ga^{3+} was 20 μM. (b) Benesi-Hildebrand plot of HT with Ga^{3+}, assuming 1:1 stoichiometry between HT and Ga^{3+}.

7. The absorption titration of HT

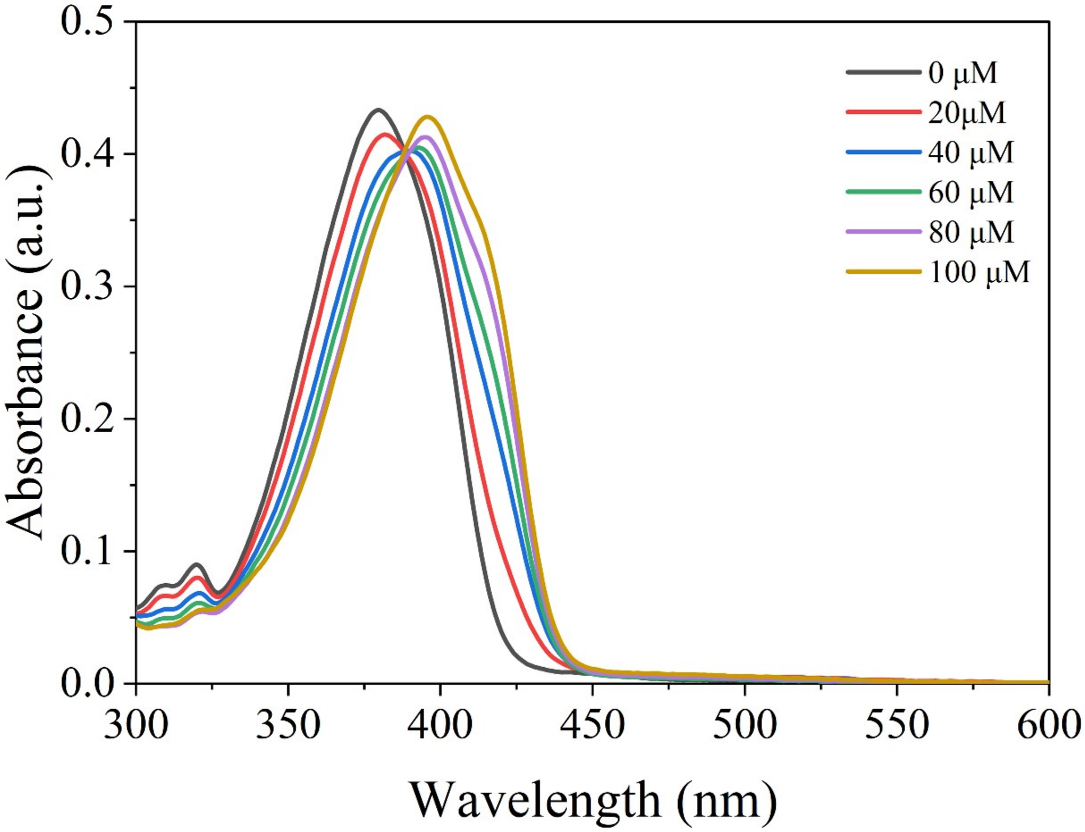
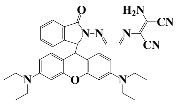
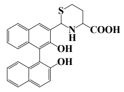
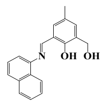
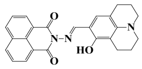
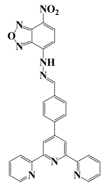
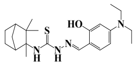


Fig. S10 The absorption titration of HT with various concentrations of Ga^{3+} in the H_2O/DMSO (v/v = 3/7).

8. Comparison of HT with other reported Ga^{3+} fluorescent probes

Table S1 Comparison of other reported Ga^{3+} fluorescent probes

Probe	Medium	Sensing mode	LOD (μM)	Interference	Bioimaging	Ref
	CH_3OH/H_2O (v/v = 1/1)	Turn-On	3.52 nM	Hg^{2+}	Cell imaging	Ind. Eng. Chem. Res., 2019, **58**, 18456-18467.
	C_2H_5OH/H_2O (v/v = 98 / 2)	Turn-On	2.37 μM	Al^{3+}, In^{3+}	No	Chem. Commun. 2016, **52**, 827-830.
	C_2H_5OH/THF (v/v = 99.5/ 0.5)	Turn-On	5.96 μM	Al^{3+}	No	Inorg. Chem., 2019, **58**, 10364-10376.
	CH_3OH	Turn-On	No	In^{3+}	No	Photochem. Photobiol. Sci., 2018, **17**, 1247-1255.
	DMSO/H2O (v/v = 9 / 2)	Turn-On	0.041	Cr^{3+}	Cells Zebrafish	Analyst, 2019, **144**, 3807-3816
	DMSO/H2O (v/v = 7/3)	Turn-On	0.0321	No	Cells	This work

9. pH-dependent fluorescence intensity

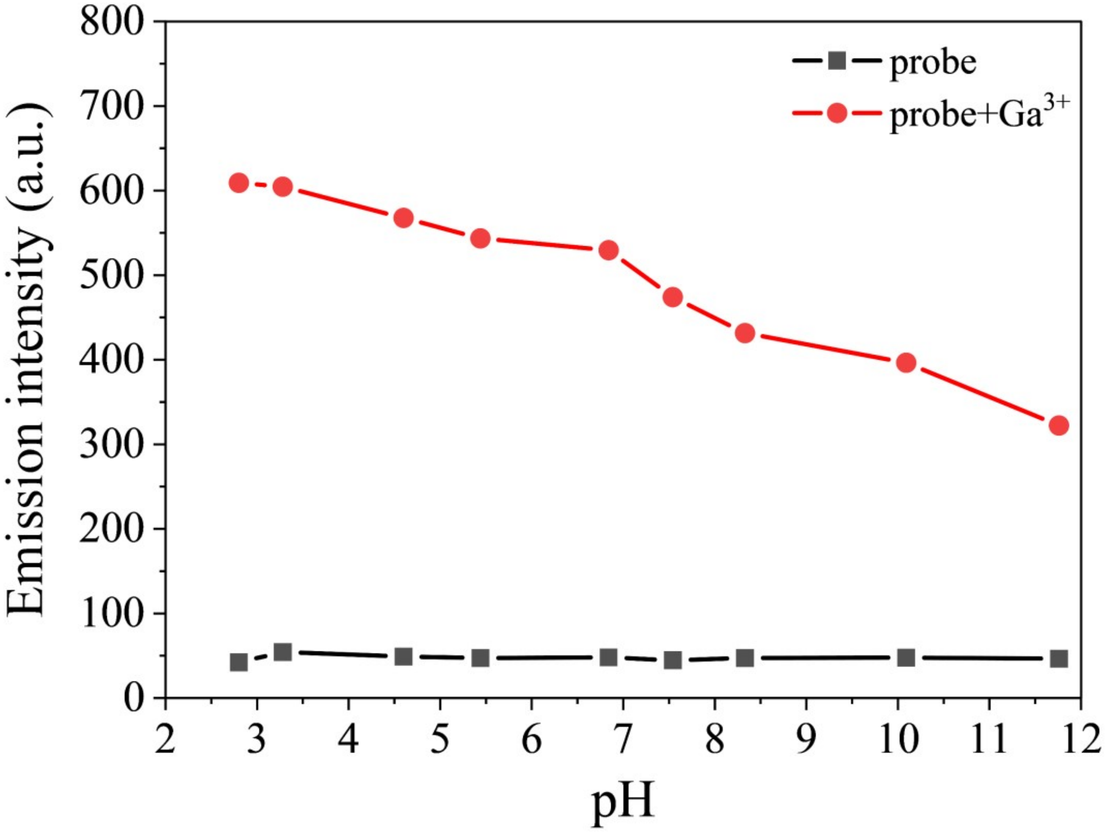


Fig. S11 Fluorescence intensity at 447 nm of HT (10 μM) toward Ga^{3+} (100 μM) at different pH values.

10. Time-dependent fluorescence intensity

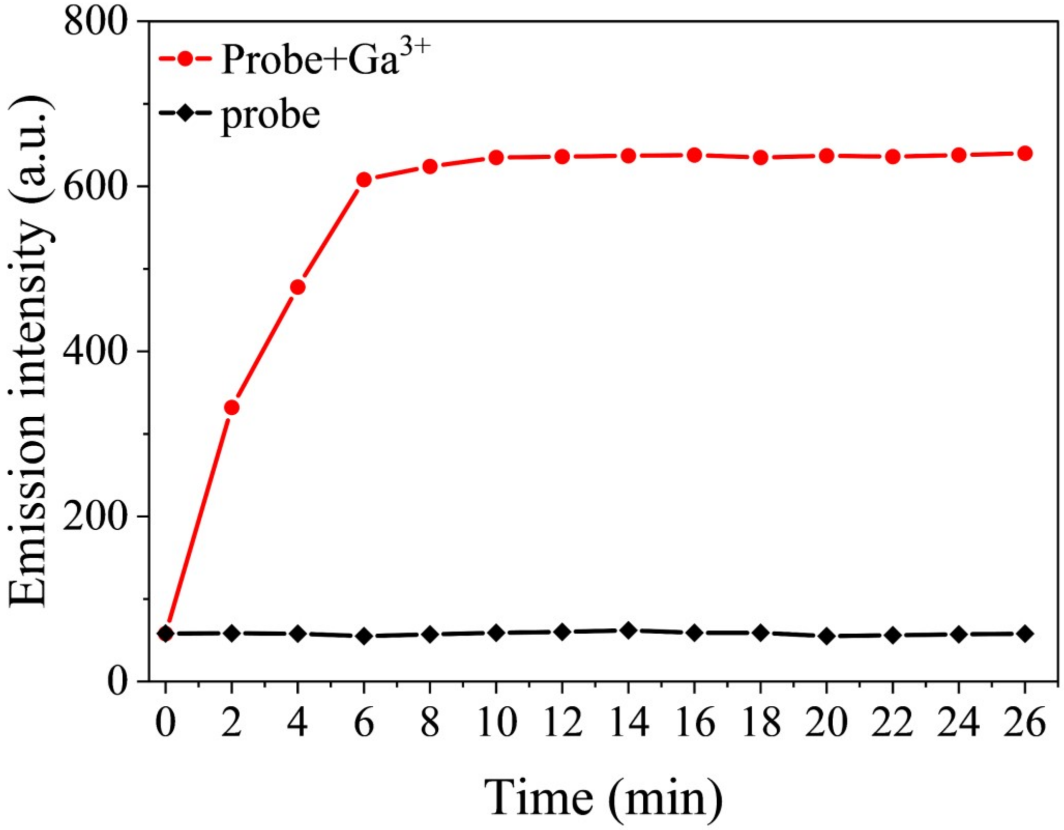


Fig. S12 The variation trend of fluorescence intensity of probe at 447 nm overtime after the addition of Ga^{3+} (10 equiv).

11. HRMS of HT with Ga^{3+}

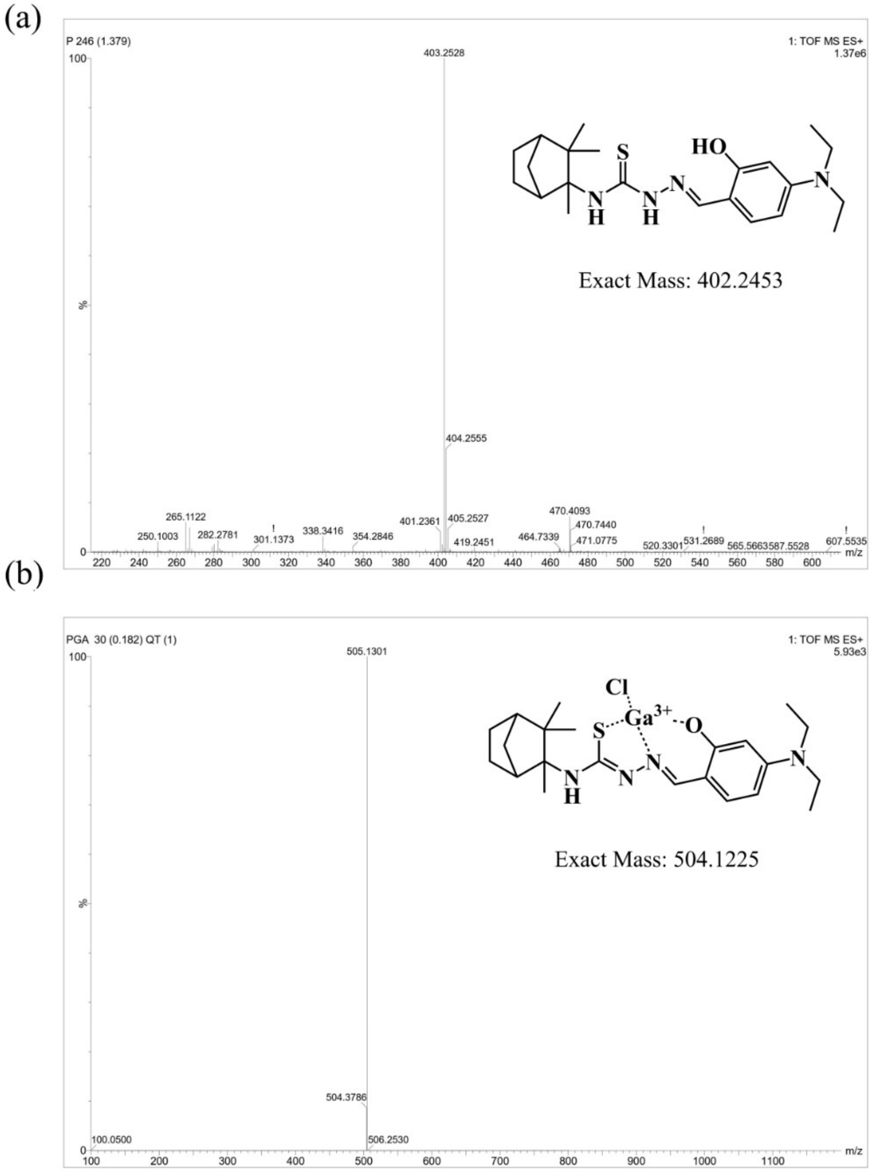


Fig. S13 (a) HRMS of HT in HPLC-methanol. (b) HRMS of HT upon addition of GaCl$_3$ in HPLC-methanol.

12. DFT calculations

Table S2 Results from TDDFT studies on HT and HT-Ga^{3+}

Compound	**Electronic transition**	**Energy (eV)**	λ（**nm**）	***f*[a]**	**Transitions involved**
HT	S_0♑S_1	3.4556 eV	358.78 nm	1.2149	HOMO->LUMO (97%)
HT-Ga^{3+}	S_0♑S_2	3.0322 eV	408.89 nm	1.0375	HOMO->LUMO (3%) HOMO->LUMO+1 (93%)

[a]Oscillator strength.

13. Application of portable test strips

We prepared the test strips by fully immersing filter paper in an EtOH solution of HT (100 μM) for 30 min to ensure that the probe can evenly distribute over filter paper strips. After that, the filter paper strips were taken out and dried in a vacuum, and the test strips were successful. Such test strips were used for on-site detection by adding aqueous solutions of various metal ions. Subsequently, after spraying various metal ion solutions (1 mM) and different concentrations of Ga^{3+}, fluorescence changes of test paper were observed and record under a 365 um UV lamp and fluorescence spectrophotometer.

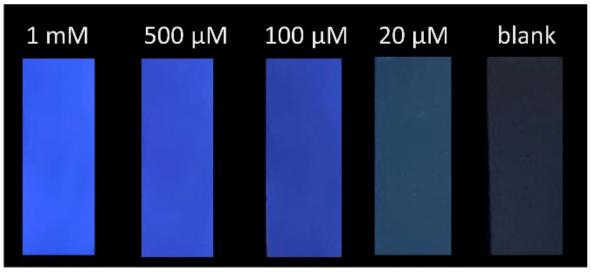


Fig. S14 Fluorescent photographs of HT-loaded test strips with different concentrations of Ga^{3+} under a 365 nm UV lamp.

14. Application in real water samples

Two types of real water samples (tap water and Yangtze River) were collected to study the practical application of probe HT. The sample of tap water was collected from our lab and directly used. Yangtze River was filtrated through filter paper and a 0.22 μM microfiltration membrane to remove impurities. Firstly, a blank experiment was carried out to check the presence of Ga^{3+} in the real water sample, the result was found to be negative. A standard addition method was used for further investigation by the addition of different concentrations of Ga^{3+} into the water samples.

The real water samples spiked with Ga^{3+} stock solution was mixed with DMSO in a volume ratio of 3:7, respectively, to prepare the tested sample solution. Subsequently, the stock solution of probe HT (100 mM, 10 uL) was added to 10 mL of the aforementioned tested sample solution with Ga^{3+}, and then the mixture solutions were analyzed by the proposed fluorescence assays. The fluorescence spectra were obtained and the fluorescence intensity at 447 nm was recorded. The Ga^{3+} concentration was obtained according to the working curve between the fluorescence intensity and the Ga^{3+} concentration of the standard addition, and the recovery rate is calculated through comparison with the theoretical value.

Table S3 Determination results of Ga^{3+} concentration in water samples by using HT

Samples	Added (μM)	Detected (μM)	RSD （%，*n*=3）	Recovery (%)
Tap water	0	No detected	-	–
	15	14.04±0.71	5.0	93.6
	30	28.71±1.09	3.8	95.7
	60	58.50±2.90	4.9	97.5
Yangtze River water	0	No detected	-	–
	15	13.08±0.54	4.1	87.2
	30	28.05±1.33	4.7	93.5
	60	55.56±2.02	3.6	92.6

15. MTT assay for cell cytotoxicity of HT

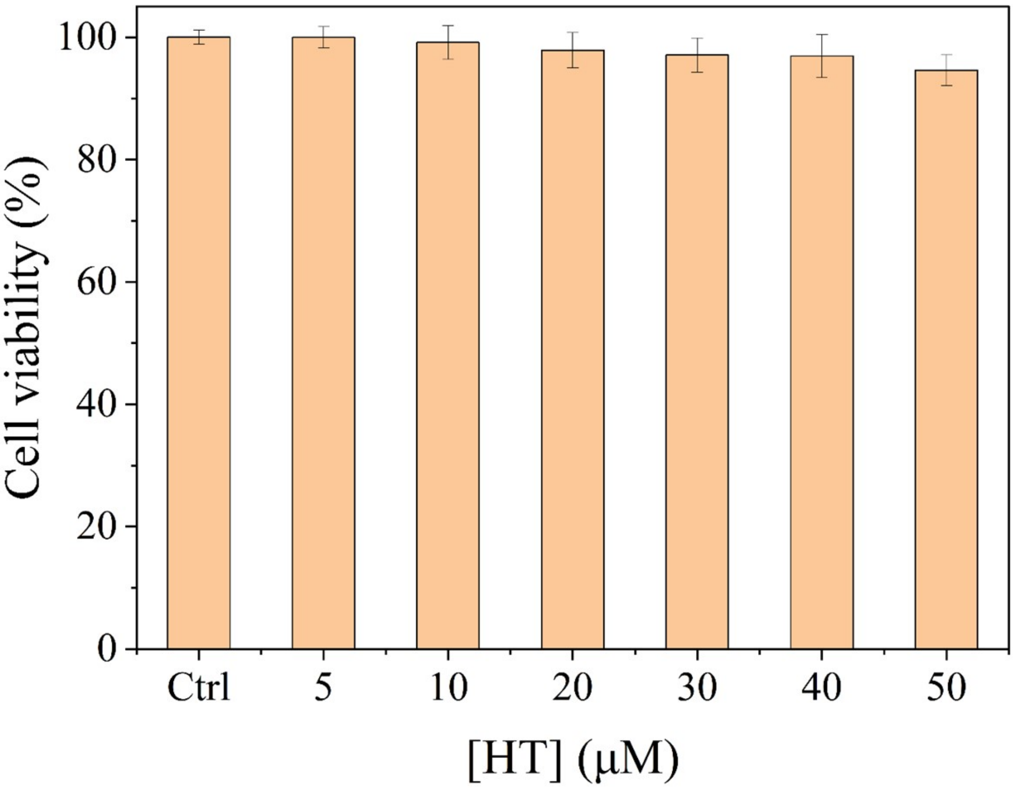


Fig. S15 Evaluation of the cell viability using MTT assay in MCF-7 cells incubate with different concentrations of HT.

16. Quantum yields measurements

The fluorescence quantum yield was determined by the relative comparison by using quinine sulfate as a standard according to the following equation

$$\Phi_s = \Phi_r (A_r F_s / A_s F_r)(\eta_s^2 / \eta_r^2)$$

in which Φ_s is the quantum yield, F_s and F_r are the corresponding relative integrated intensity of the emission spectra, A_s and A_r are the absorbance of the sample and reference solutions at the excitation wavelength, respectively, and η is the respective refractive indices of the solution, and the subscripts s and r refer to the sample and the reference, respectively.