Supporting Information

A near-infrared fluorescence turn-on probe based on Michael addition-intramolecular cyclization for specific detection of cysteine and its applications in environment water, milk and living cells

Zhigang Gao[a], Ling Zhang [b], Minchuan Yan[a], Haibo Liu[a], Shaohui Lu[a], Huihui Lian[a], Peng Zhang[b], Jing Zhu[b,*], Mingjie Jin[a,*]

[a] School of Environmental and Biological Engineering, Nanjing University of Science and Technology, 200 Xiaolingwei, Nanjing 210094, P. R. China

[b] Department of Pharmacy, Nanjing University of Chinese Medicine, 138 Xialin Dadao, 210023, P. R. China

[*] Corresponding authors

Email: 830640@njucm.edu.cn; jinmingjie@njust.edu.cn

Contents

Materials and instruments

All chemicals were purchased from commercial companies and were used without further purification unless specified. All solvents were of analytical grade, and double-distilled water was used in all of the experiments. Data of ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured on Bruker Avance 500 spectrometer. Mass spectra were obtained by Thermo Finnigan TSQ Quantum LC/MS Spectrometer. The pH levels of stock solutions were measured using a pH meter (Denver UB-7). UV–vis absortion spectra and fluorescence spectra were recorded by Hitachi U-3900 and Fl3-TCSPC fluorescence spectrophotometer, respectively. High performance liquid chromatography (HPLC) experiments were recorded on a Thermo Ultimate 3000 with a Thermo Acclaim 120 C18 column (5 μm, 4.6 × 250 mm).

Pretreament of real water samples and milk samples

The real water samples were collected from the tap water in our laboratory and Xuanwu Lake (Nanjing, China). The water samples were filtered by aqueous microporous membrane before recording spectra. The water samples were used to prepare MeCN/H_2O (1:1, v/v) solutions, and pH values of these solutions were adjusted to 7.4. Then different concentrations of Cys (0, 15, 20, 30, 40, 50, 60, 70, 80 μM) were added in the MeCN/H_2O solutions, and then added **RHI** (10 μM). The resulting solutions were shaken well and incubated for 20 min at 37 ℃.

The milk samples were purchased from a supermarket (Nanjing, China). The milk

samples were diluted 100 times by using deionized water. The milk samples were used to prepare MeCN/milk (1:1, v/v) solutions, and pH values of these solutions were adjusted to 7.4. Then different concentrations of Cys (0, 20, 30, 40, 50, 60 μM) were added in the MeCN/milk solutions, followed by addition of **RHI** (10 μM). The resulting solutions were shaken well and incubated for 20 min at 37 ℃ before recording the spectra.

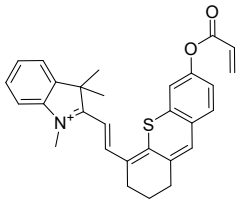
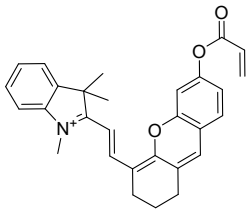
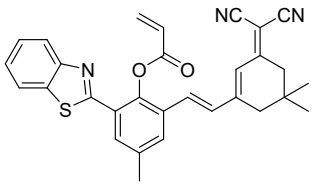
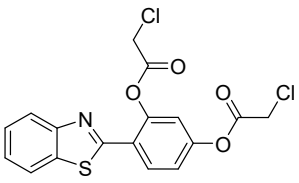
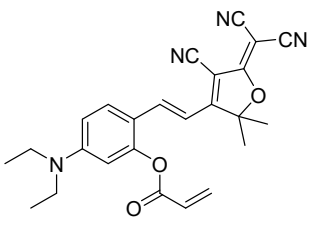
Cytotoxicity assay and fluorescence imaging

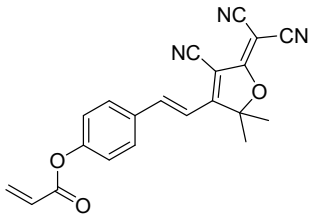
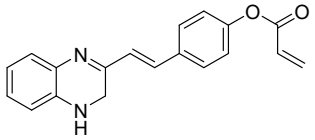
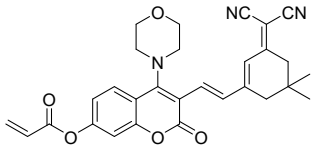
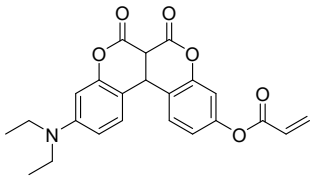
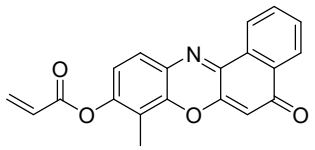
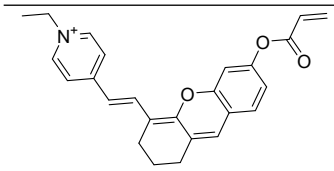
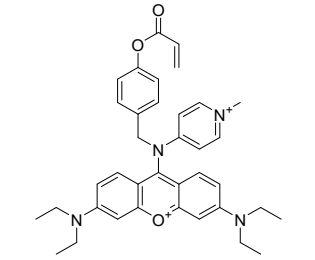
The MTT method was used to investigate the cytotoxicity of probe **RHI**. Firstly, HeLa cells were incubated with a density of 9×10^3 cells in a 96-well plate and incubated in an atmosphere of 5% CO_2 / 95% air at 37 °C for 24 h. Subsequently, different concentrations (0, 1.25, 2.5, 5, 10, 20 μM) of probe **RHI** were added into the cells, and then cultured at 37 °C for 24 h. Finally, MTT (5 mg/mL) was incubated for another 4 h. The cell survival rate was with a microplate reader (Tecan Infinite 200 PRO).

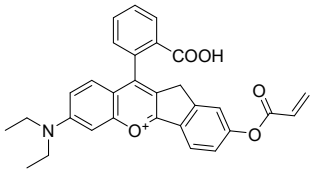
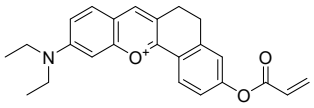
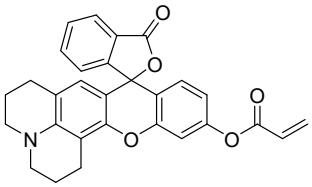
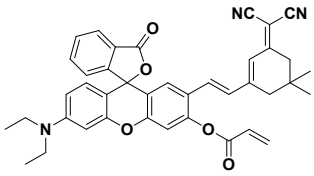
HeLa cells were cultured at 37 °C in a 5% CO_2/95% air incubator, and cultured in Dulbecco's modified Eagle medium containing 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin. The cells were cultured on a 24-well plate for 12 hours. In fluorescence imaging experiments, HeLa cells were first treated with **RHI** probe (10 μM) and incubated at 37°C for 30min. Then the cells were incubated with NEM (biological sulfhydryl blocker N-ethylmaleimide, 0.5 mm) at 37°C for 60min, **RHI** (10 μM) at 37°C for another 30min. Finally, the NEM (0.5 mM, 60 min) pretreated cells were incubated with different concentrations of Cys (10, 50 μM) for another 30

min, and then probe **RHI** (10 μM) for 30 min at 37 °C. Living cells imaging experiments were performed in an inverted fluorescence microscopy (Olympus, Japan). Fluorescence images were collected with emissions at 650-750 nm under excitation at 580 nm.

Table **S1**. The comparison between the probe in this work and those in previous reports.

Probe	Stokes shift (nm)	Response time (min)	LOD (μM)	Application	Reference
	42	5	0.016	Cells	[1]
	27	5	0.160	Cells	[2]
	263	28	0.98	Cells, mice	[3]
	55	-	0.32	Cells	[4]
	90	30	0.13	Cells, *C. elegans*	[5]

	77	12	0.04	Cells, zebrafishes	[6]
	64	120	1.0	Cells	[7]
	170	5	0.053	Cells	[8]
	75	20	0.411	Cells, tissues	[9]
	47	15	0.019	Cells	[10]
	136	7	0.0489	Cells, mice	[11]
	15	30	0.033	Cells	[12]

	61	15	0.024	Cells	[13]
	45	30	0.12	Cells	[14]
	29	14	0.0392	Cells	[15]
	155	15	0.168	Cells, real water and milk samples	**This work**

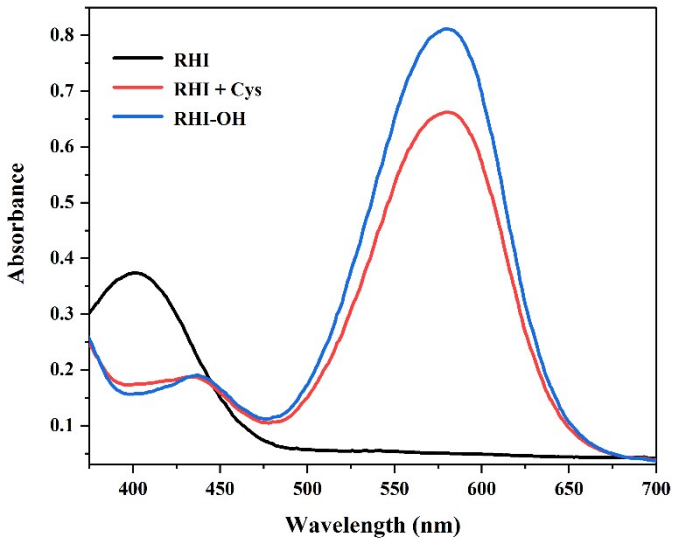


Fig. S1. Absorption spectra of probe **RHI** (10 μM) before and after addition of Cys (50 μM), and **RHI-OH** (10 μM) in MeCN-PBS buffer solution (1:1, v/v, 10 mM, pH=7.4).

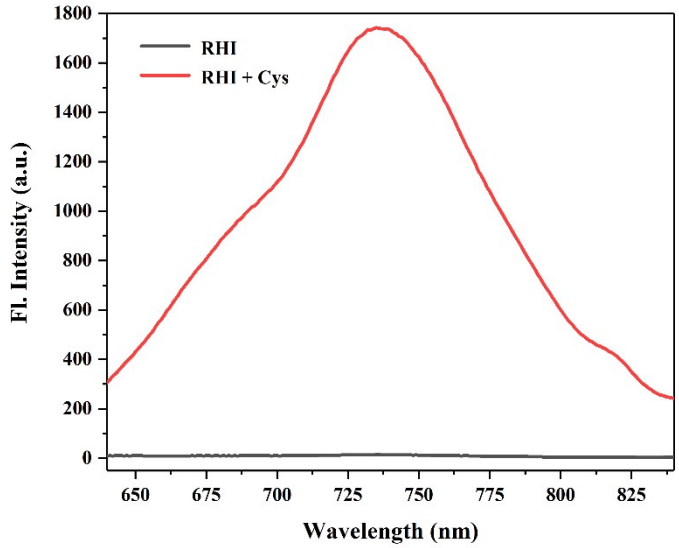


Fig. S2. Absorption spectra of probe **RHI** (10 μM) before and after addition of Cys (50 μM) in MeCN-PBS buffer solution (1:1, v/v, 10 mM, pH=7.4).

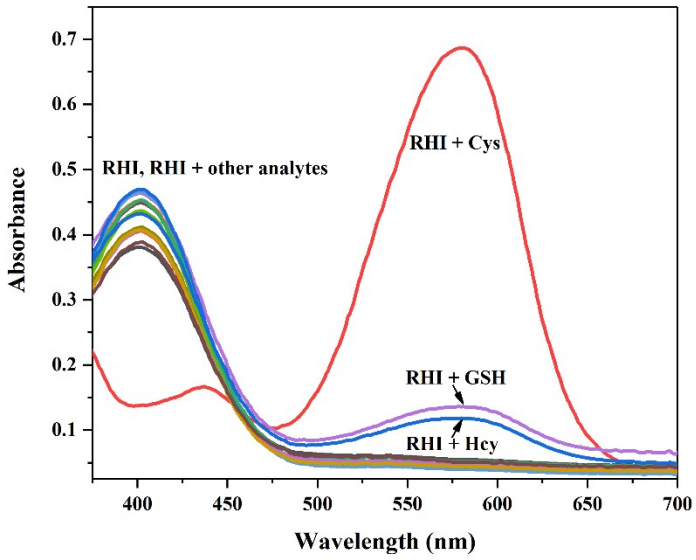


Fig. S3. UV–vis absorption spectra of probe **RHI** (10 μM) toward various relevant analytes (Cys, Hcy, GSH, Asp, Val, Phe, Tyr, Ser, Ala, Leu, Pro, Arg, Hyp, Glu, Thr, Gly, Trp, Ile, His, Met, Lys, Try, HS^-, S^{2-}, SO_3^{2-}, SO_4^{2-}, Mg^{2+}, Zn^{2+}, 50 μM for each) in MeCN-PBS buffer solution (1:1, v/v, 10 mM, pH=7.4).

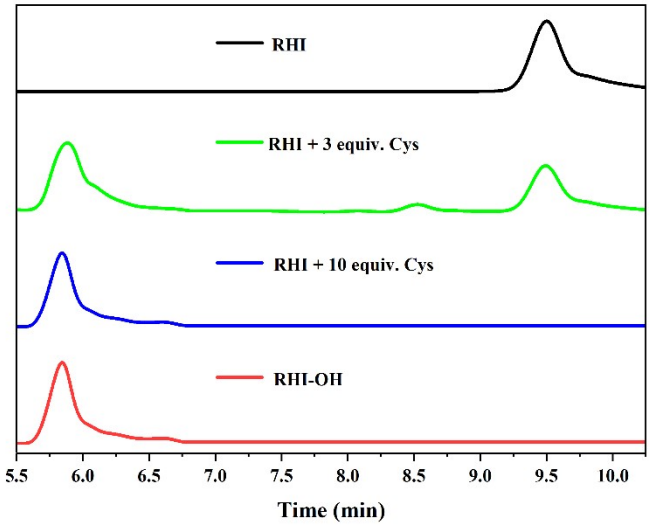


Fig. S4. HPLC chromatogram analysis of **RHI** (10 μM, 1.0 equiv.), **RHI** (1.0 equiv.) + 3.0 equiv. Cys, **RHI** (1.0 equiv.) + 10.0 equiv. Cys, **RHI-OH** (10 μM). Conditions: 1.0 mL/min flow rate; Thermo Acclaim 120 C18: 5μm, 4.6 × 250 mm column; mobile phase: acetonitrile/ PBS = 80:20; detection wavelength: 335 nm; injection volume: 20 μL.

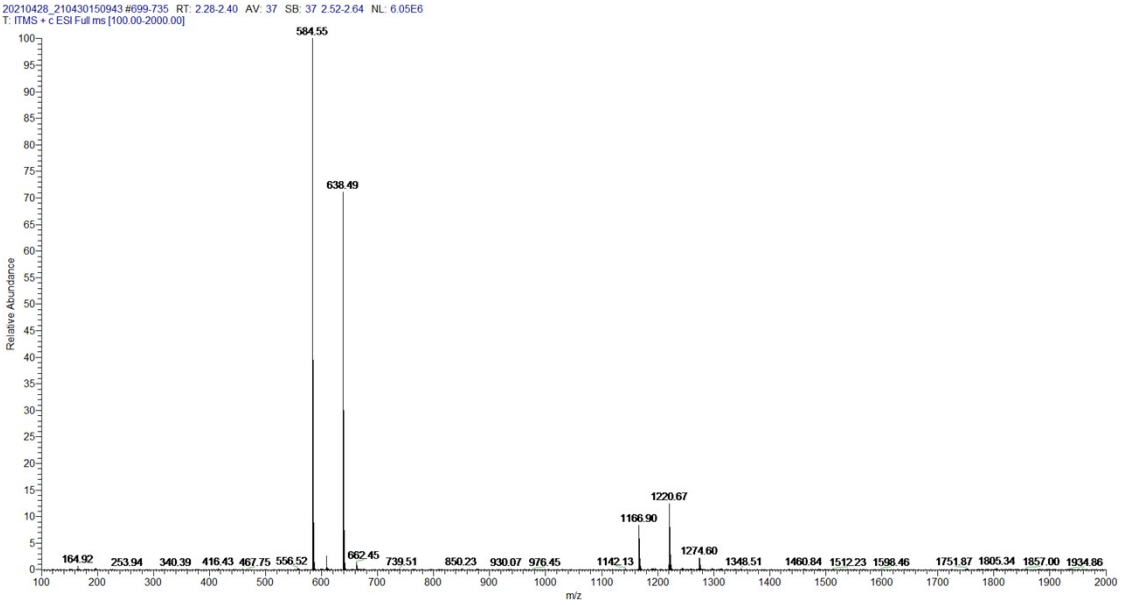


Fig. S5. MS spectrum of **RHI** after addition of Cys.

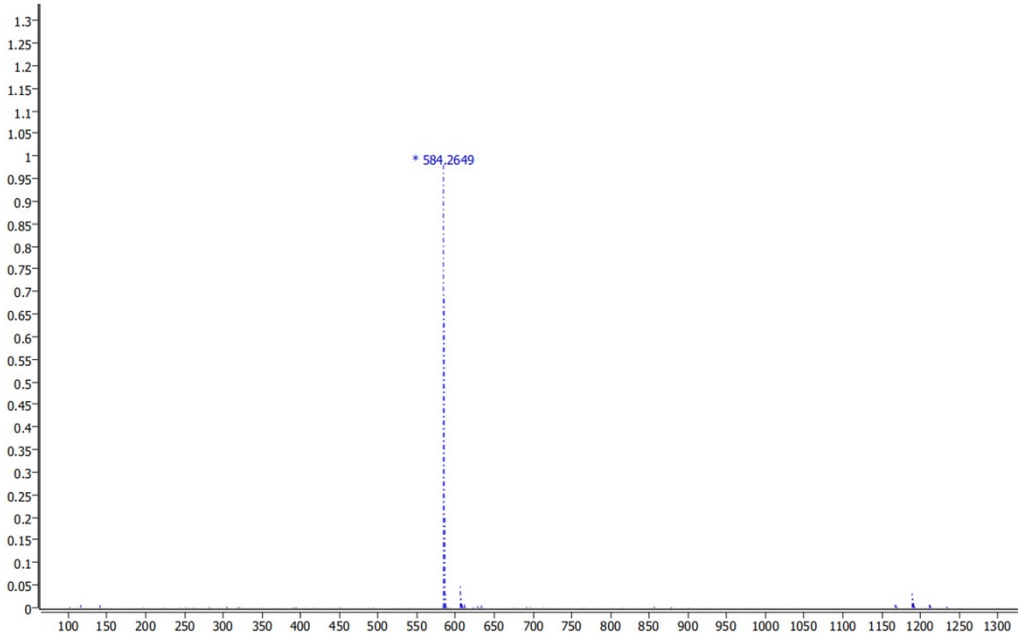


Fig. S6. HRMS spectrum of the reaction product of **RHI** with Cys.

Table S2. Determination of Cys in real water samples using **RHI** (10 μM).

Spiked (μM)	Tap water			Lake water		
	Founded (μM)	Recovery (%)	RSD (%, n=3)	Founded (μM)	Recovery (%)	RSD (%, n=3)
0	-	-	-	-	-	-
15.0	15.04	100.3	2.78	14.93	99.5	2.04
20.0	19.96	99.8	1.18	19.81	99.1	2.28
30.0	29.89	99.6	2.64	30.94	103.1	4.01
40.0	41.61	104.0	3.04	41.02	102.5	1.22
50.0	51.09	102.2	2.28	52.25	104.5	3.26
60.0	60.34	100.6	2.84	63.50	105.8	2.69
70.0	71.39	102.0	2.47	69.77	99.7	0.91
80.0	79.68	99.6	3.54	80.32	100.4	4.07

Table S3. Determination of Cys in milk samples using **RHI** (10 μM)

Spiked (μM)	Milk A			Milk B		
	Founded (μM)	Recovery (%)	RSD (%, n=3)	Founded (μM)	Recovery (%)	RSD (%, n=3)
0	20.36	-	1.84	20.77	-	0.65
20.0	41.91	103.8	2.32	41.40	101.5	1.69
30.0	50.40	100.1	2.14	50.63	99.7	1.64
40.0	63.13	104.6	0.79	63.40	104.3	1.99
50.0	69.93	99.4	2.99	72.57	102.5	2.29
60.0	78.77	98.0	1.73	80.58	99.8	4.99

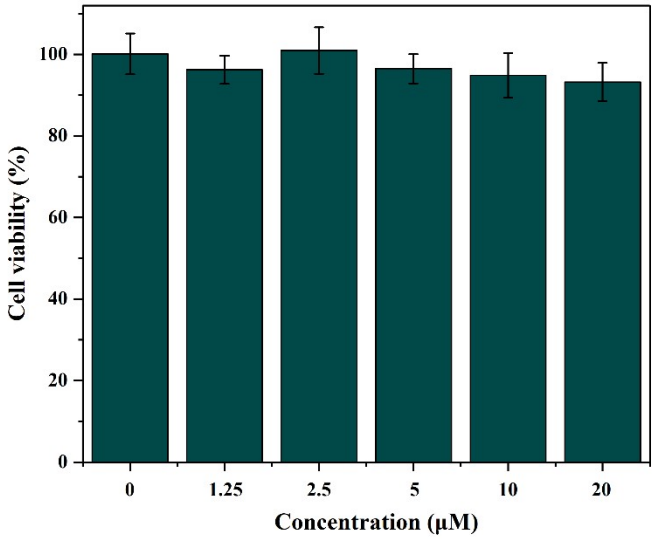


Fig. S7. Cytotoxicity studies of probe **RHI** by the standard MTT assays.

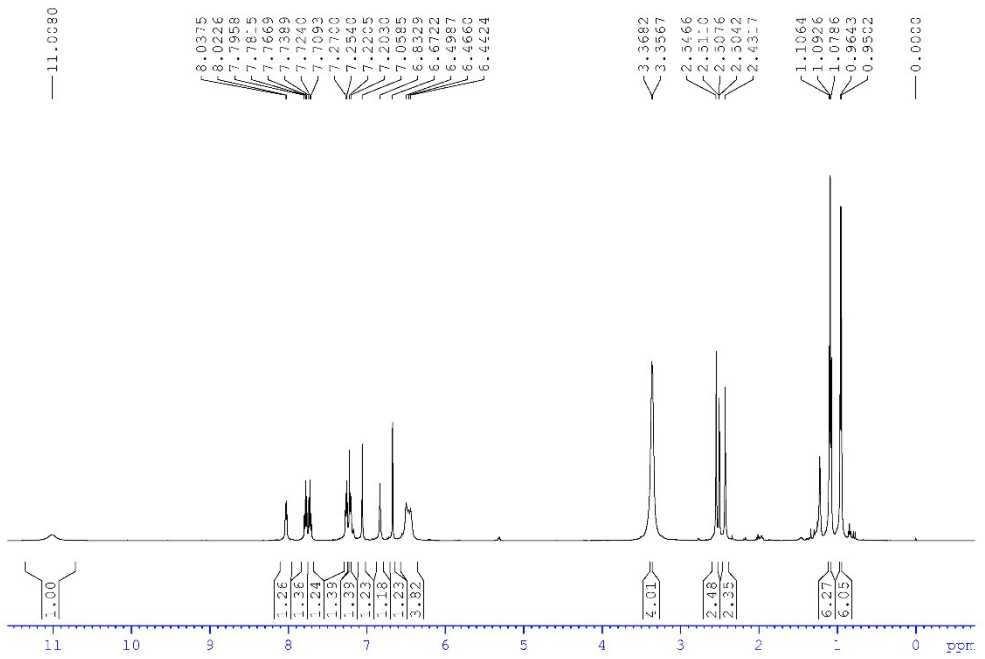


Figure S8. [1]H-NMR spectrum of **RHI-OH**.

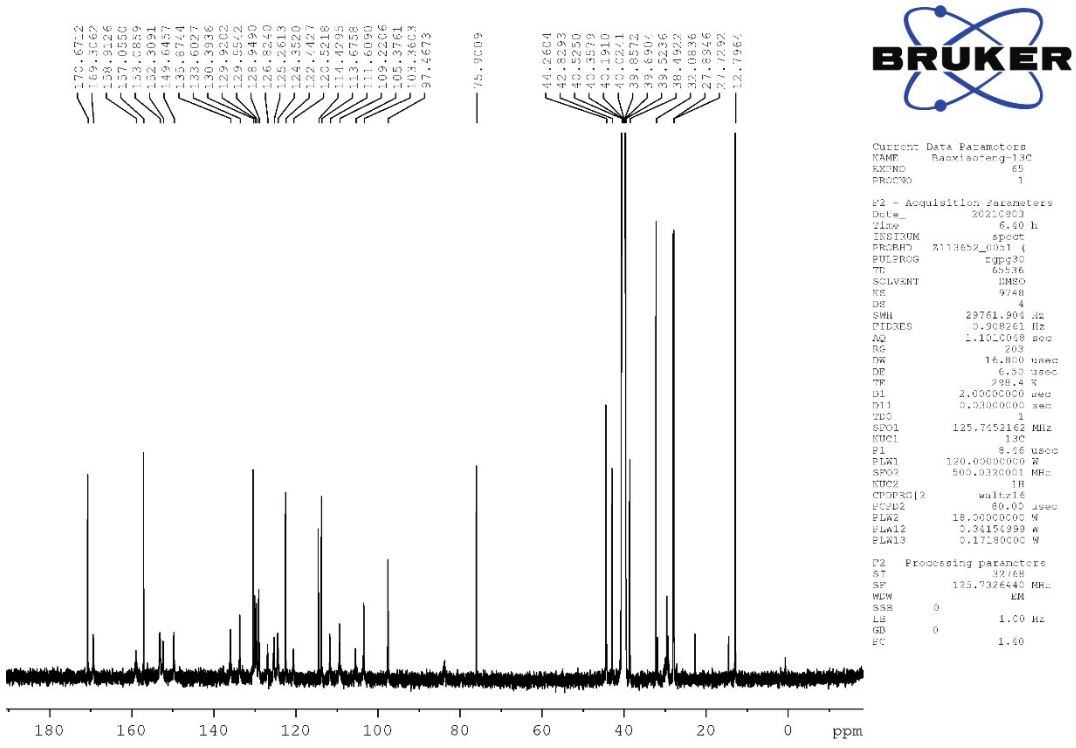


Figure S9. [13]C-NMR spectrum of **RHI-OH**.

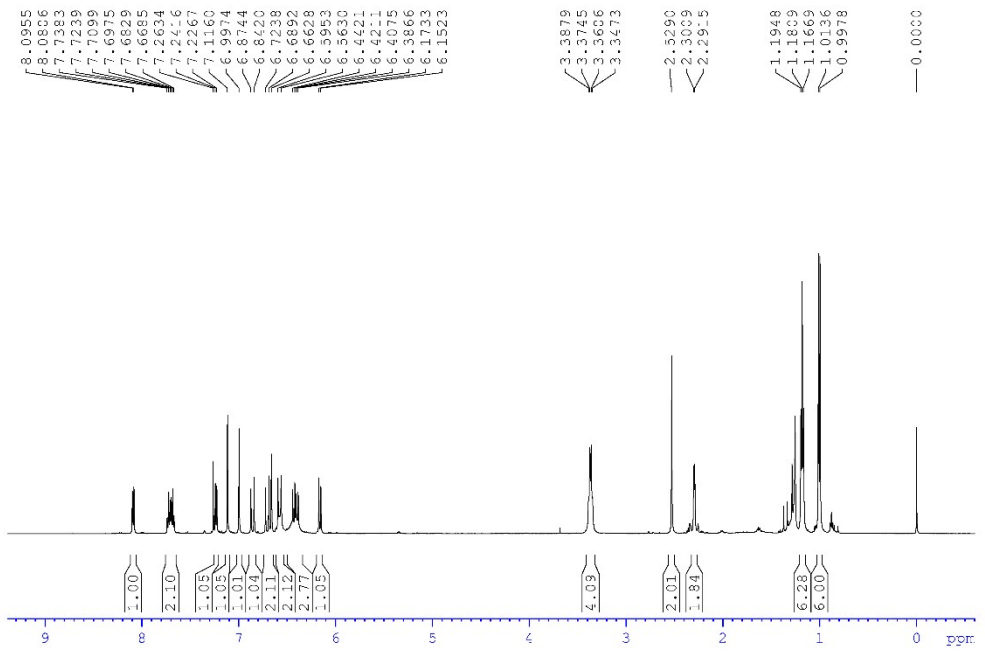


Figure S10. [1]H-NMR spectrum of **RHI**.

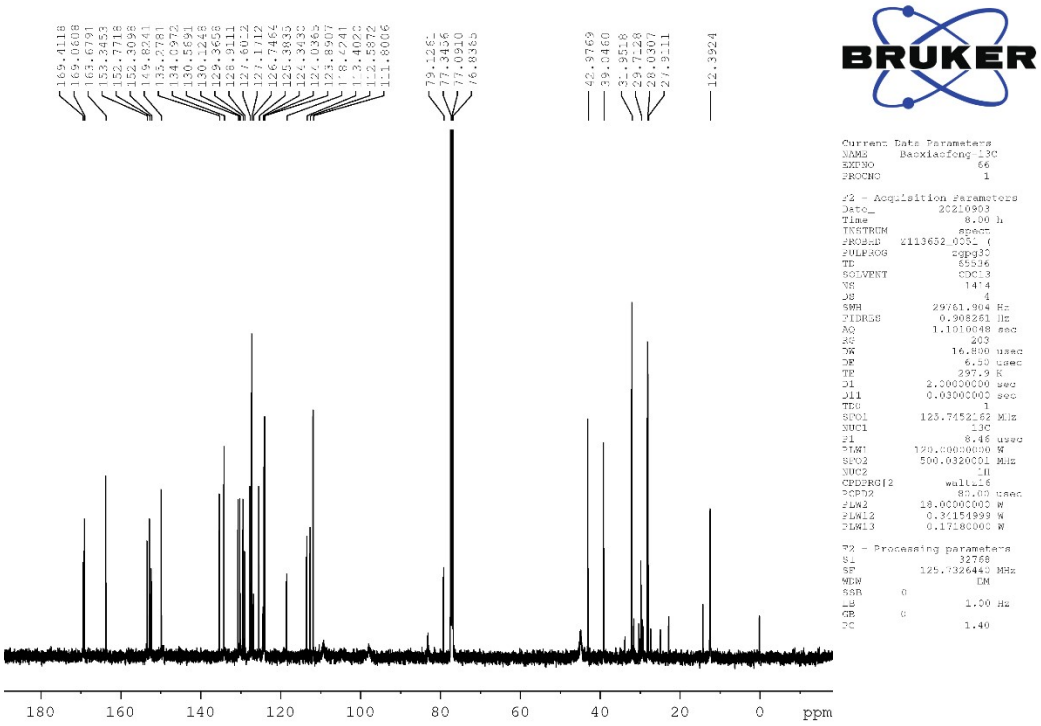


Figure S11. [13]C-NMR spectrum of **RHI**.

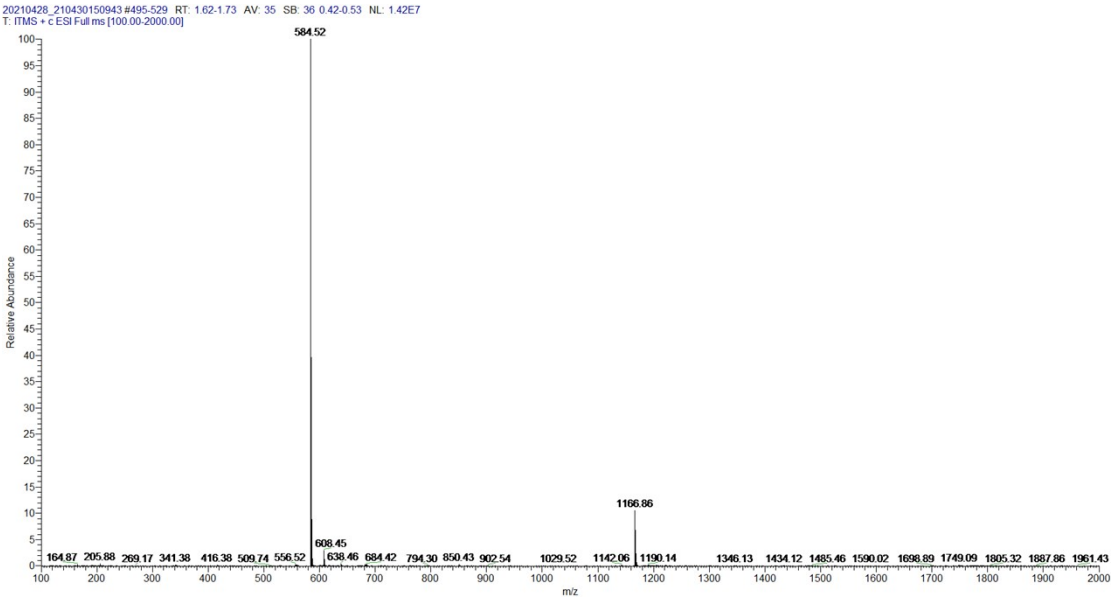


Figure S12. MS spectrum of **RHI-OH**.

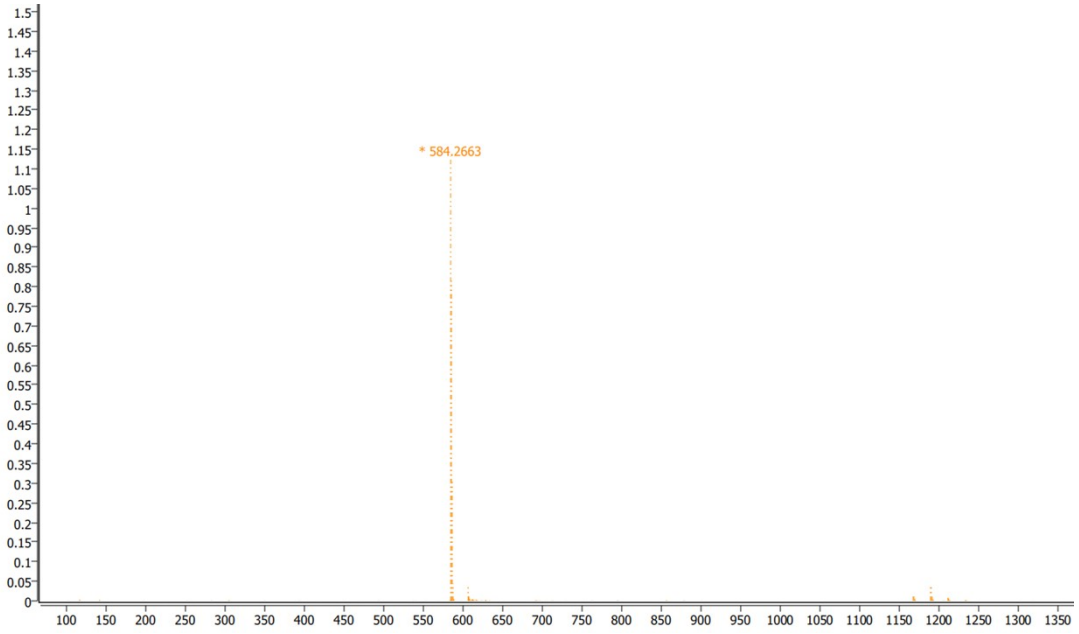


Figure S13. HRMS spectrum of **RHI-OH**.

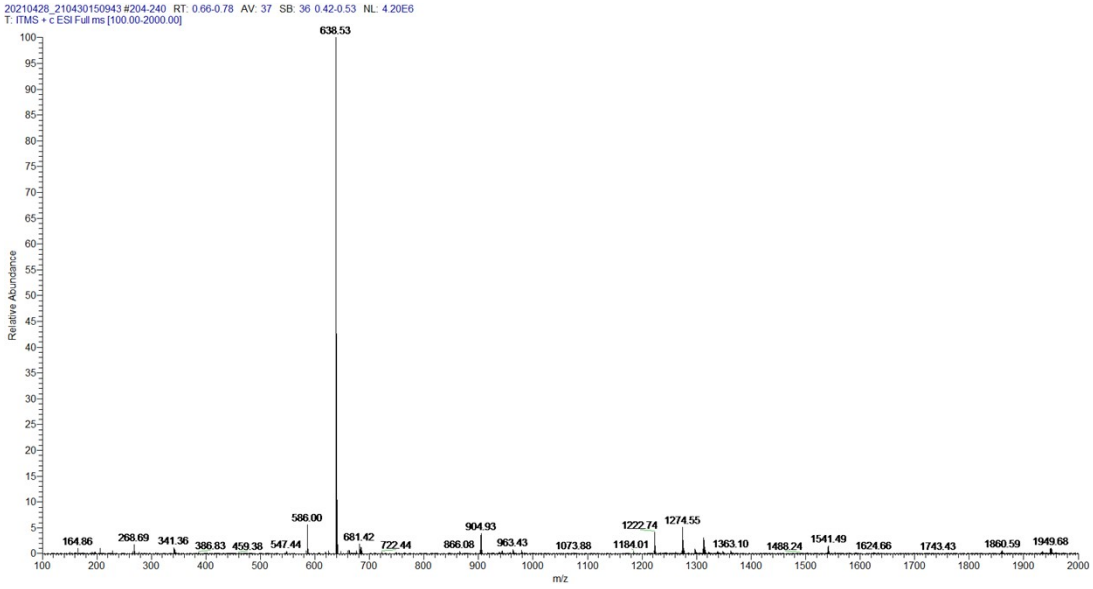


Figure S14. MS spectrum of **RHI**.

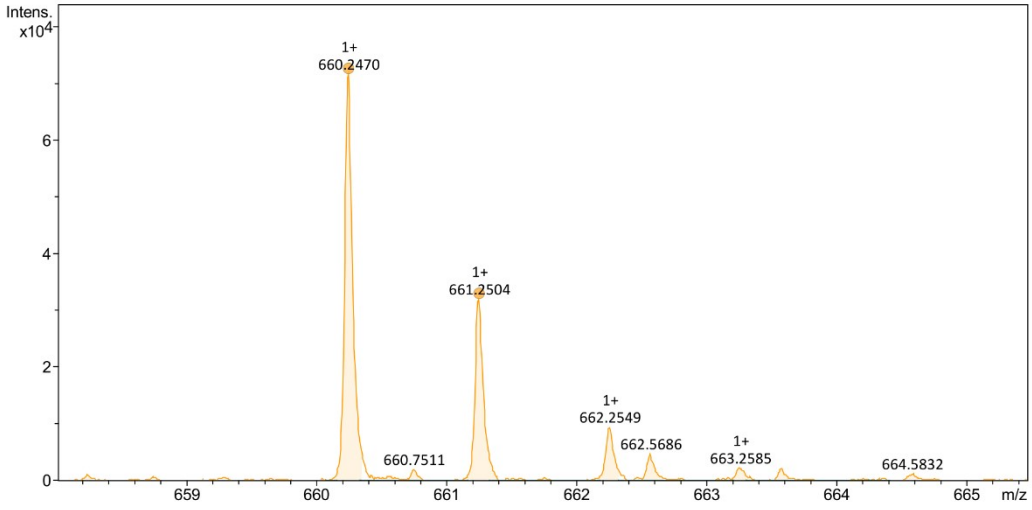


Figure S15. HRMS spectrum of **RHI**.

Reference

1 S. Cai, C. Liu, X. Jiao, L. Zhao, X. Zeng, *J. Mater. Chem. B*, 2020, **8**, 2269-2274.

2 J. Zhang, J. Wang, J. Liu, L. Ning, X. Zhu, B. Yu, X. Liu, X. Yao, H. Zhang, *Anal. Chem*., 2015, **87**, 4856-4863.

3 H. Ren, F. Huo, Y. Zhang, S. Zhao, C. Yin, *Sens. Actuators, B*., 2020, **319**, 128248.

4 Y. Yu, H. Xu, W. Zhang, B. Wang, Y. Jiang, *Talanta*, 2018, **176**, 151-155.

5 K. Wang, W. Wang, M. Guo, S. Chen, Y. Yang, B. Wang, C. Xu, H. Zhu, *Anal. Chim. Acta*, 2021, **1152**, 338243.

6 B. Zhang, H. Zhang, M. Zhong, S. Wang, Q. Xu, D. Cho, H. Qiu, *Chinese Chem. Lett*., 2020, **31**, 133-135.

7 B. Yang, J. Xu, Z. Yuan, D. Zheng, Z. He, Q. Jiao, H. Zhu, *Talanta*, 2018, **189**, 629-635.

8 Y. Zhang, Y. Zhang, Y. Yue, J. Chao, F. Huo, C. Yin, *Sens. Actuators, B*., 2020, **320**, 128348.

9 C. Chen, L. Zhou, W. Liu, W. Liu, *Anal. Chem*., 2018, **90**, 6138-6143.

10 X. Yang, X. Wei, R. Sun, Y. Xu, J. Ge, *Spectrochim. Acta*., 2020, **226**, 117582.

11 Y. Dai, Y. Zheng, T. Xue, F. He, H. Ji, Z. Qi, *Spectrochim. Acta*., 2020, **225**, 117490.

12 X. Yang, X. Wei, R. Sun, Y. Xu, J. Ge, *Talanta*, 2020, **209**, 120580.

13 D. Liu, Y. Lv, M. Chen, D. Cheng, Z. Song, L.Yuan, X. Zhang, *J. Mater. Chem. B*, 2019, **7**, 3970-3975.

14 S. Jiao, X. He, L. Xu, P. Ma, C. Liu, Y. Huang, Y. Sun, X. Wang, D. Song, *Sens. Actuators, B*., 2019, **290**, 47-52.

15 Y. Ji, F. Dai, B. Zhou, *Talanta*, 2019, **197**, 631-637.