

Electronic Supplementary Information

A phenothiazine-based ratiometric fluorescence probe for the detection of hydroxylamine in real water and living cells

Man Du^a, Haohua Jiang^a, Meimei Song^a, Yue Zhang^{a,*}, Haijun Lv^a, Shuchun

Zhao^a, Hongxia Du^a, Zhipeng Dong^{b,**}

^aCollege of Chemical and Pharmaceutical Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, P. R. China;

^bHebei Lansheng Bio-Tech Co., Ltd, Shijiazhuang, 052263, China

*Corresponding author

**Corresponding author

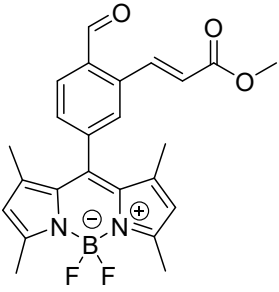
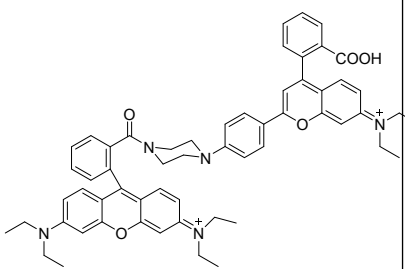
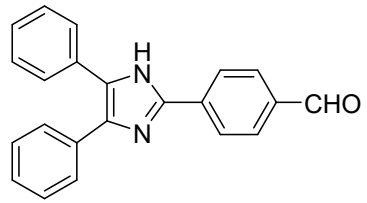
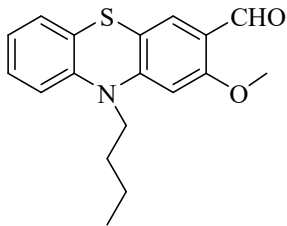
E-mail addresses: yuezhang@hebust.edu.cn (Yue Zhang), dzpkjcx@sohu.com

(Zhipeng Dong)

Contents

Table S1 Comparison of fluorescent probes for hydroxylamine (HA).	3
Fig. S1 ¹ H NMR spectrum of compound 2-Methoxy-10-butyl-10H-phenothiazine (1) in CDCl ₃	4
Fig. S2 ¹³ C NMR spectrum of compound 2-Methoxy-10-butyl-10H-phenothiazine (1) in CDCl ₃	4
Fig. S3 HRMS spectrum of compound 2-Methoxy-10-butyl-10H-phenothiazine (1). .	5
Fig. S4 ¹ H NMR spectrum of the probe PCHO in CDCl ₃	5
Fig. S5 ¹³ C NMR spectrum of the probe PCHO in CDCl ₃	6
Fig. S6 HRMS spectrum of the probe PCHO	6
Fig. S7 ¹ H NMR spectrum of PCHO-HA in CDCl ₃	7
Fig. S8 ¹³ C NMR spectrum of PCHO-HA in CDCl ₃	7
Fig. S9 HRMS spectrum of the probe PCHO in absence (a) and presence of HA (b). .	8
DFT Calculation of the probe PCHO	9
DFT Calculation of PCHO-HA	11
Fig. S10 Cytotoxicity assays of the probe PCHO at different concentrations for HUVEC cells.	13
References	14

Table S1 Comparison of fluorescent probes for hydroxylamine (HA).

Probes	$\lambda_{\text{ex}}/\lambda_{\text{em}}$ (nm)	LOD (μM)	Linear range (μM)	Application	Ref.
	465/510	—	0~20	—	[1]
N-CDs/ Fe^{3+}	360/477	0.246	0~100	—	[2]
	540/637	2.16	0~80	Imaging in living cells	[3]
DNA-CuNPs	340/588	0.022 mM	0.1-1.2 mM	Detection in real water samples	[4]
	365/465	28	0~1000	—	[5]
	$\lambda_{\text{ex}}=380$ nm; $\lambda_{\text{em1}}=519$ nm; $\lambda_{\text{em2}}=478$ nm;	0.19	0~1000	Test strip with Smartphone; Detection in real water samples; Imaging in living cells	This work

“—“ Not mentioned.

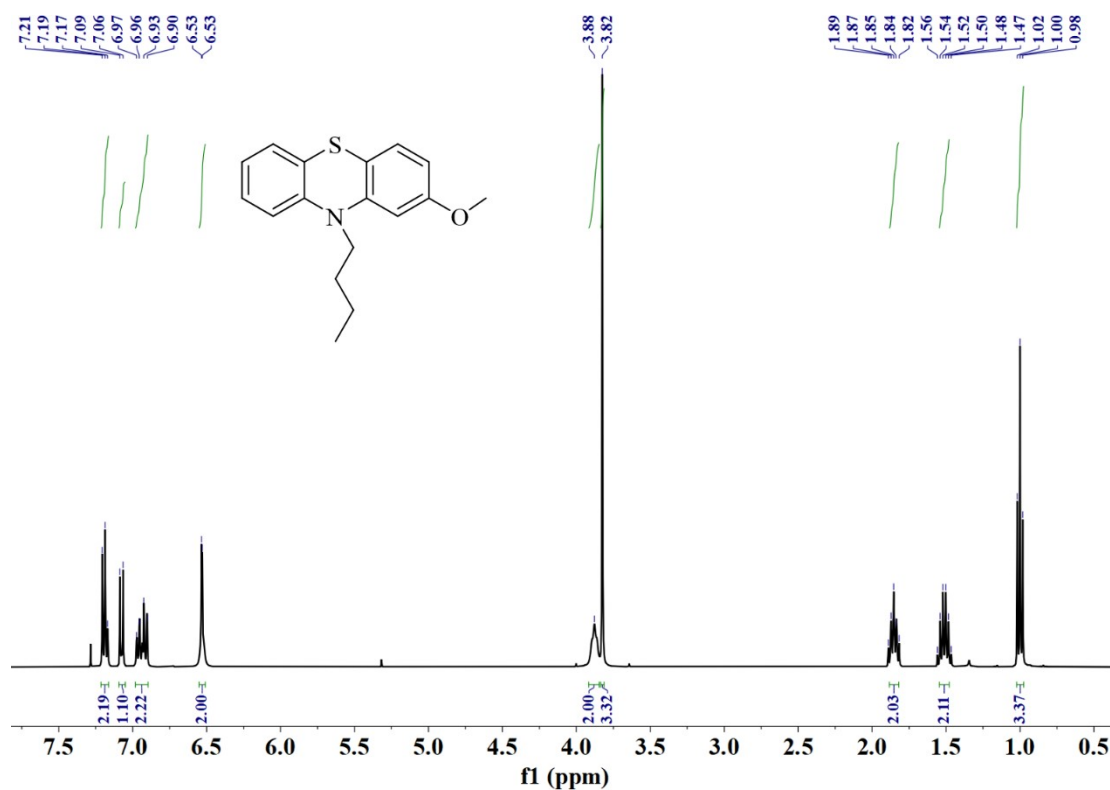


Fig. S1 ¹H NMR spectrum of compound 2-Methoxy-10-butyl-10H-phenothiazine (**1**) in CDCl₃.

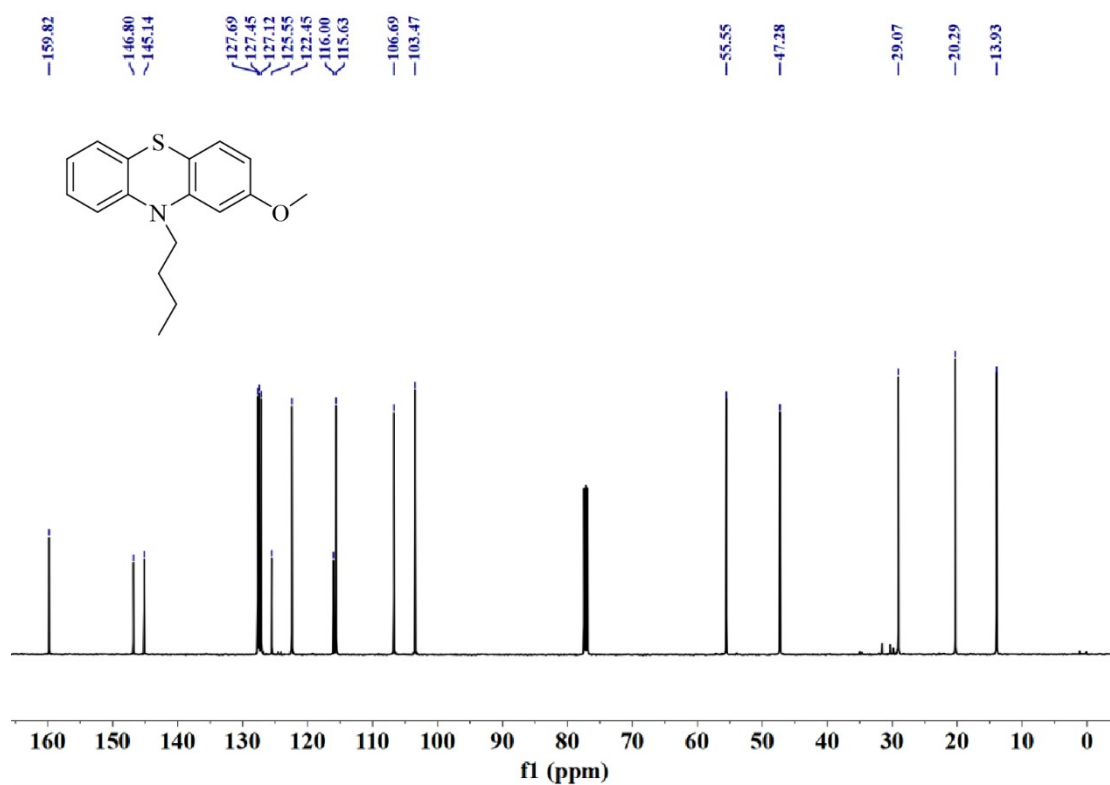


Fig. S2 ¹³C NMR spectrum of compound 2-Methoxy-10-butyl-10H-phenothiazine (**1**) in CDCl₃.

probe-1_20240619105602 #12 RT: 0.06 AV: 1 SB: 40 0.00-0.04 , 0.14-0.32 NL: 1.49E9
T: FTMS + p ESI Full ms [160.0000-2000.0000]

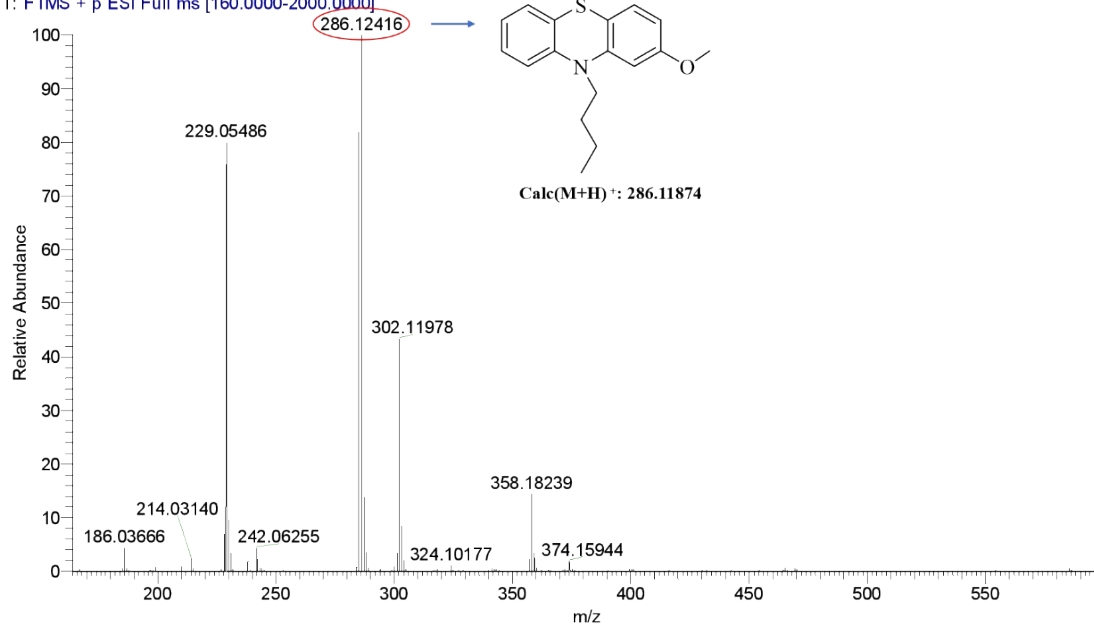


Fig. S3 HRMS spectrum of compound 2-Methoxy-10-butyl-10H-phenothiazine (**1**).

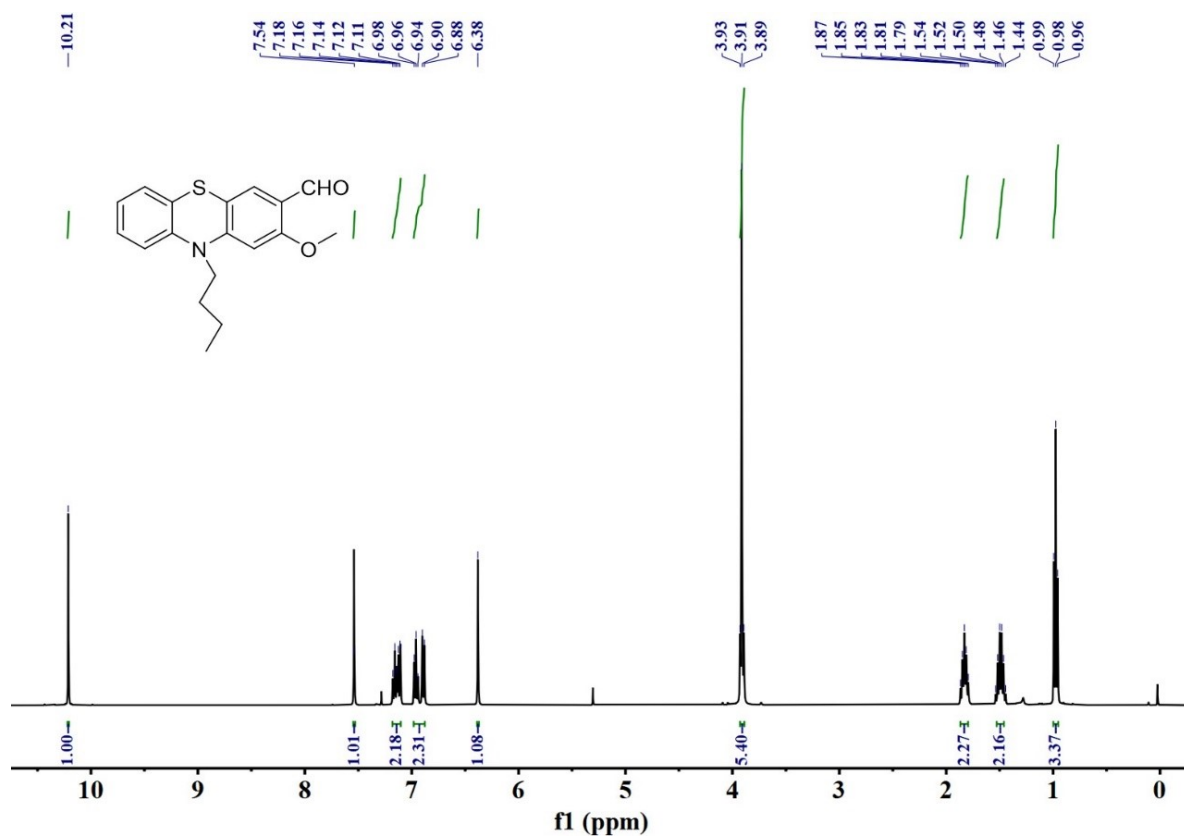


Fig. S4 ¹H NMR spectrum of the probe PCHO in CDCl₃.

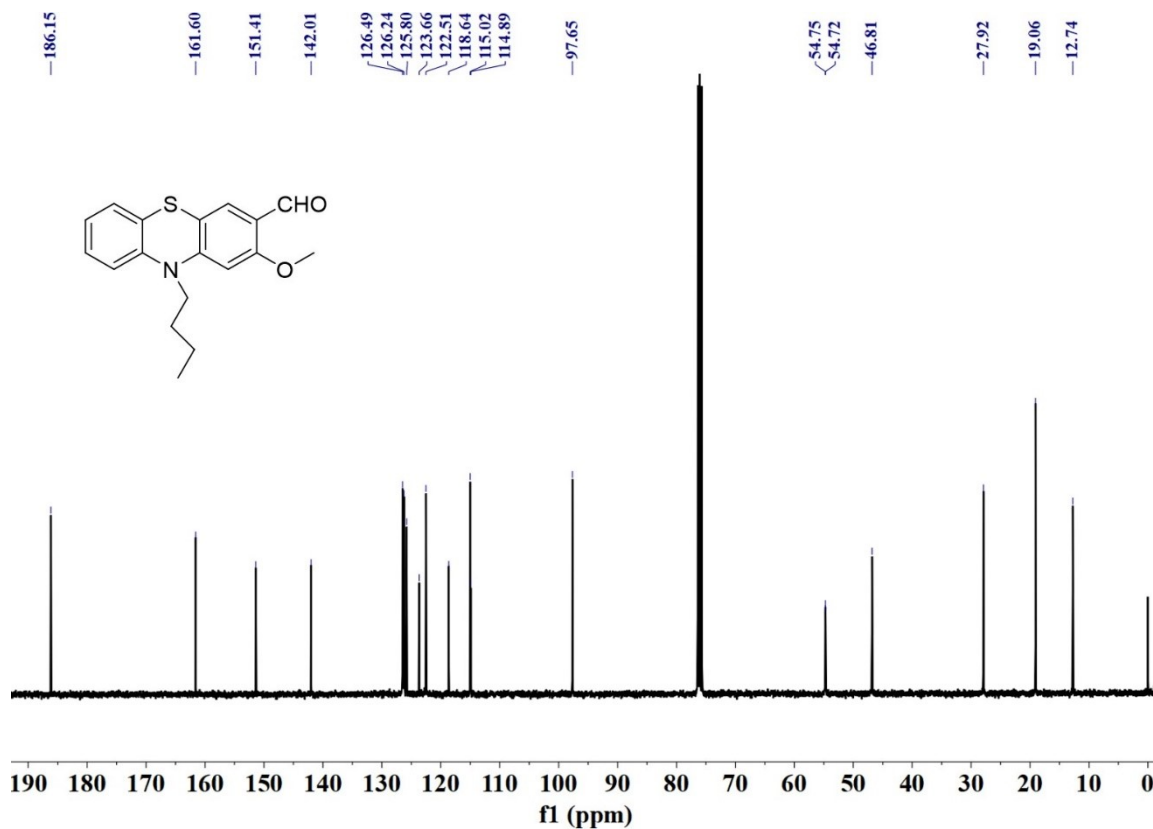


Fig. S5 ¹³C NMR spectrum of the probe PCHO in CDCl₃.

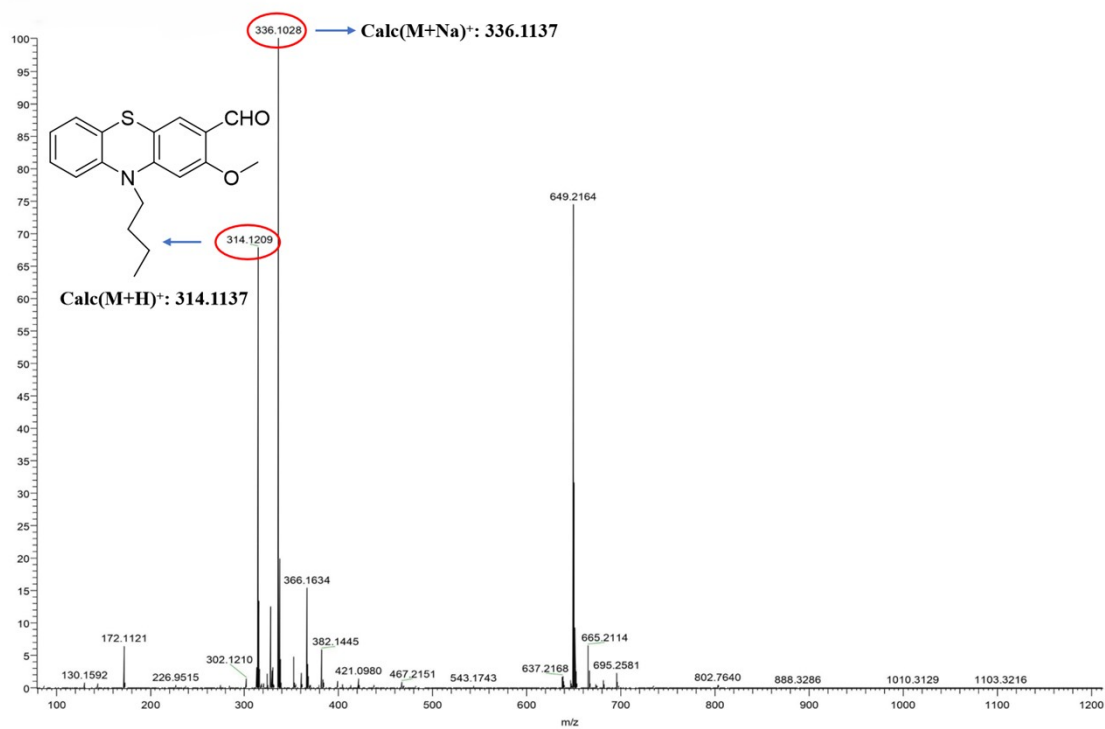


Fig. S6 HRMS spectrum of the probe PCHO.

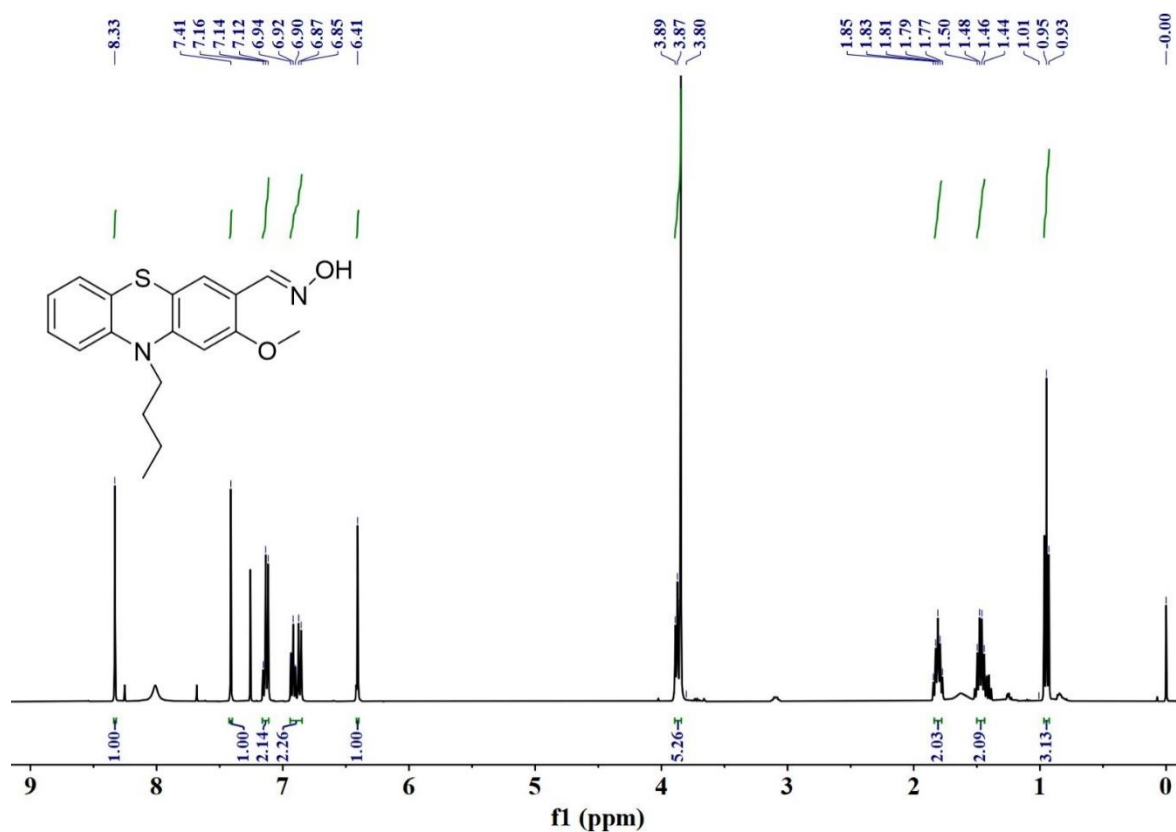


Fig. S7 ¹H NMR spectrum of PCHO-HA in CDCl₃.

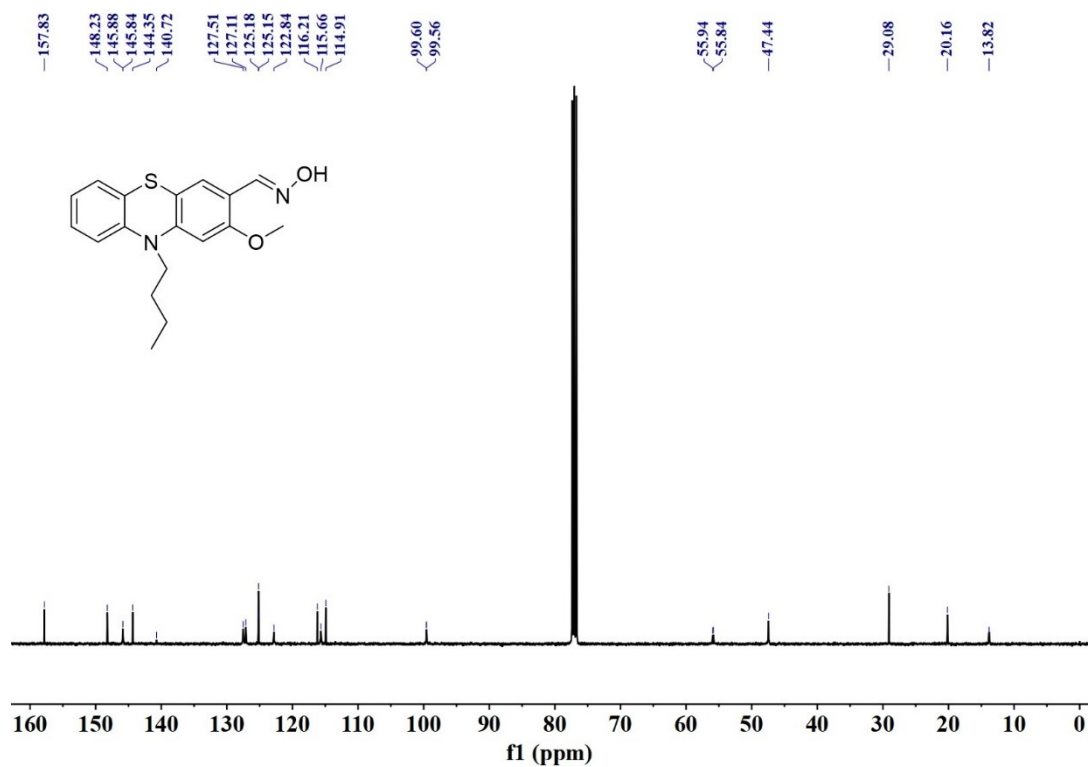


Fig. S8 ¹³C NMR spectrum of PCHO-HA in CDCl₃.

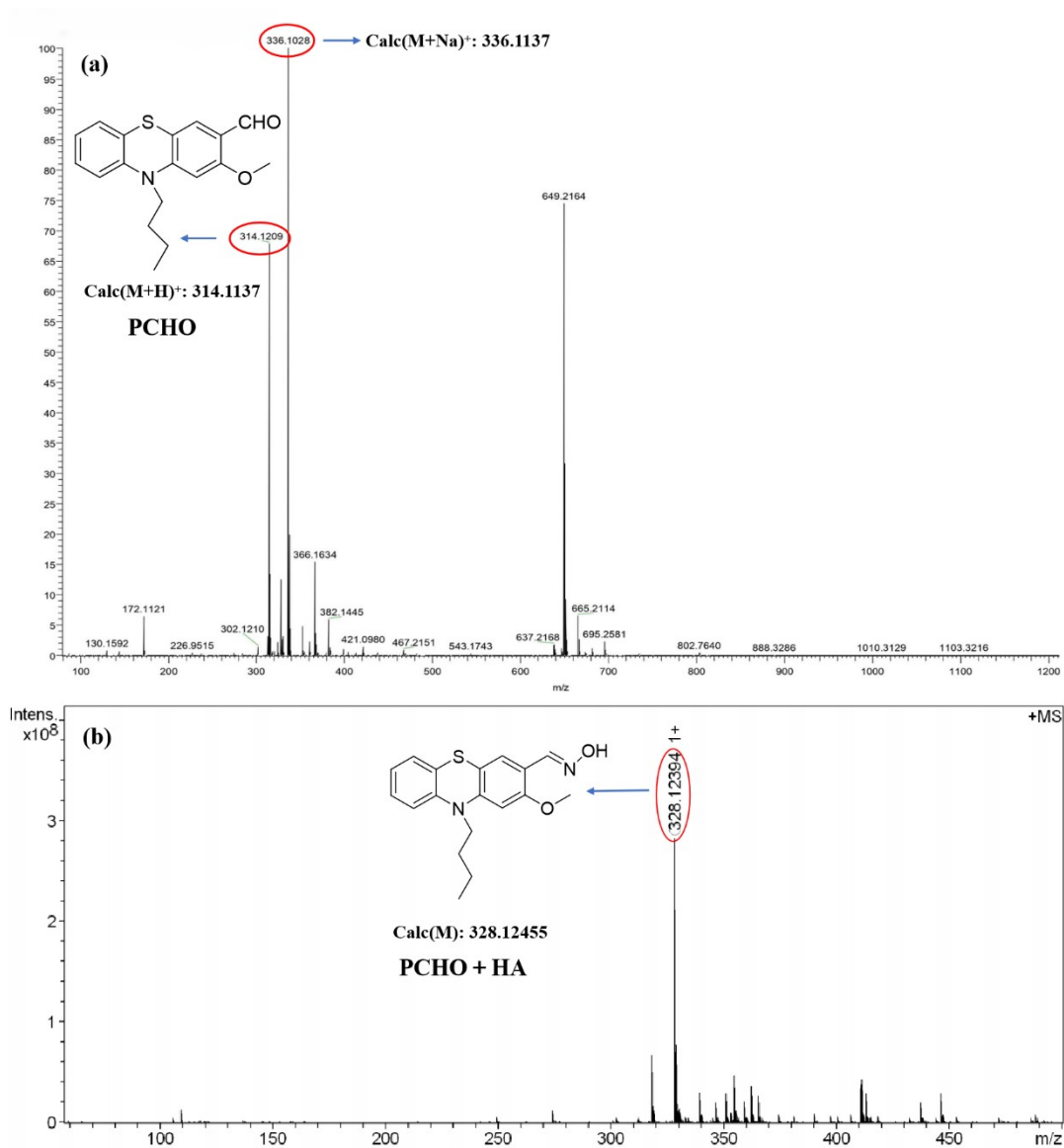


Fig. S9 HRMS spectrum of the probe **PCHO** in absence (a) and presence of HA (b)

DFT Culculation of the probe PCHO

opt freq b3lyp/6-31g geom=connectivity

• Cartesian Coordinates for **PCHO**:

C	-3.18693916	-2.70179634	0.00263100
C	-1.79177916	-2.70179634	0.00263100
C	-1.09424116	-1.49404534	0.00263100
C	-1.79189516	-0.28553634	0.00143200
C	-3.18672016	-0.28561434	0.00095300
C	-3.88432116	-1.49382034	0.00194900
H	-3.73669816	-3.65411334	0.00308100
H	-1.24227116	-3.65430934	0.00394600
H	-3.73684216	0.66666666	0.00000000
H	-4.98392516	-1.49363734	0.00176900
S	-0.90131253	1.25565234	0.00133650
N	0.37575859	-1.49393840	0.00347850
C	1.36190929	-0.06134600	0.00466102
C	2.75706929	-0.06134600	0.00466102
C	3.45460729	1.14640500	0.00466102
C	2.75695329	2.35491400	0.00346202
C	1.36212829	2.35483600	0.00298302
C	0.66452729	1.14663000	0.00397902
H	3.30657729	-1.01385900	0.00597602
H	0.81200629	3.30711700	0.00203002
C	0.96722801	-2.62321335	0.00282562
C	2.47394601	-2.62321335	0.00282562
H	0.57271501	-3.14584635	-0.90201638
H	0.57279301	-3.14571235	0.90780162
C	3.03153701	-4.03106435	0.00300362
H	2.85114001	-2.07317535	-0.89961038

H	2.84753901	-2.09262835	0.91831762
C	4.53827101	-4.03103635	0.00293862
H	2.65436201	-4.58108835	0.90542162
H	2.65429801	-4.58128935	-0.89927138
H	4.92707301	-5.07783235	0.00308762
H	4.93258201	-3.50841035	-0.90201738
H	4.93266201	-3.50811935	0.90769162
O	4.88460705	1.14650903	0.00548546
C	5.36185198	2.49452048	0.00703612
H	5.00428624	2.99846630	0.88058369
H	6.43185101	2.49405991	0.00840204
H	5.00651787	2.99968674	-0.86671712
C	3.52745736	3.68830197	0.00337940
H	4.59745404	3.68577865	0.00424234
O	2.90299229	4.78082784	0.00230049

DFT Culculation of PCHO-HA

opt freq b3lyp/6-31g geom=connectivity

• Cartesian Coordinates for **PCHO-HA**:

C	-3.81851812	-2.70179634	0.00263100
C	-2.42335812	-2.70179634	0.00263100
C	-1.72582012	-1.49404534	0.00263100
C	-2.42347412	-0.28553634	0.00143200
C	-3.81829912	-0.28561434	0.00095300
C	-4.51590012	-1.49382034	0.00194900
H	-4.36827712	-3.65411334	0.00308100
H	-1.87327412	0.66660666	0.00137300
H	-4.36842112	0.66666666	0.00000000
H	-5.61550412	-1.49363734	0.00176900
S	0.05417958	-1.49391585	0.00365723
N	-1.68878543	-3.97509908	0.00438887
C	0.82191732	-5.25095373	0.00531073
C	2.21707732	-5.25095373	0.00531073
C	2.91461532	-4.04320273	0.00531073
C	2.21696132	-2.83469373	0.00411173
C	0.82213632	-2.83477173	0.00363273
C	0.12453532	-4.04297773	0.00462873
H	0.27215832	-6.20327073	0.00576073
H	2.76716132	-1.88255073	0.00405273
C	-1.94672910	-5.13863760	-0.12343947
C	-3.39296920	-5.53253403	-0.27652221
H	-1.50773056	-5.59832981	0.79497664
H	-1.34985966	-5.47912297	-1.00399743
C	-3.55290657	-7.03402026	-0.39015023
H	-3.98040836	-5.16085481	0.60437372

H	-3.81307736	-5.05901434	-1.20307820
C	-4.99917516	-7.42789818	-0.54316886
H	-2.96549095	-7.40569214	-1.27102964
H	-3.12291006	-7.52462989	0.52284602
H	-5.09333883	-8.53757254	-0.62505230
H	-5.59595768	-7.08725678	0.33738694
H	-5.43814007	-6.96790645	-1.46147266
O	2.93166164	-6.48960878	0.00702076
C	4.33226338	-6.23249920	0.13782381
H	4.86234655	-7.16073638	0.18565245
H	4.67220112	-5.67076739	-0.70704287
H	4.51024473	-5.67361121	1.03273545
C	4.45461506	-4.04309070	0.00619858
H	4.98784603	-4.97075678	0.00681492
N	5.10576619	-2.92532365	0.00620180
O	6.08934475	-2.45119950	-0.46817205
H	6.70318490	-3.17996652	-0.35112971

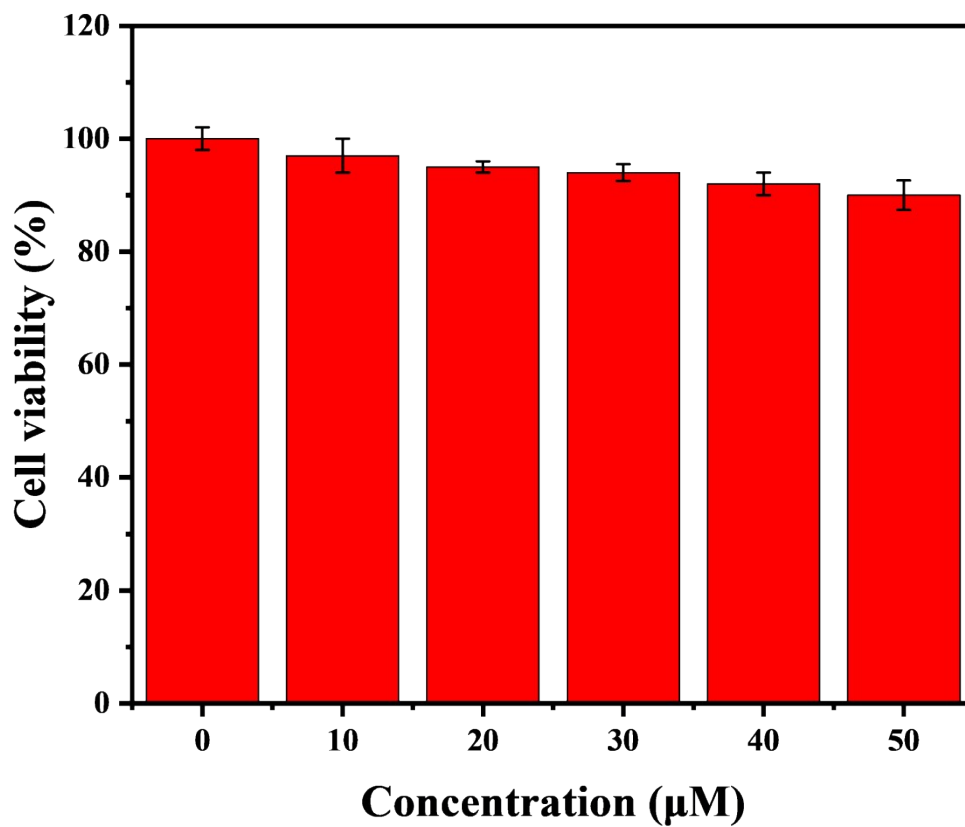


Fig. S10 Cytotoxicity assays of the probe **PCHO** at different concentrations for HUVEC cells.

References

- [1] A. C. Sedgwick, R. S. L. Chapman, J. E. Gardiner, L. R. Peacock, G Kim, J. Yoon, S. D. Bull and T. D. James, *Chem. Commun.*, 2017, **53**, 10441-10443.
- [2] J. Y. Wu, Y. Dong, X. Y. Yang and C. Yao, *Opt. Mater.*, 2019, **94**, 121-129.
- [3] B. L. Dong, M. G. Tian, X. Q. Kong, W. H. Song, Y. R. Lu and W. Y. Lin, *Anal. Chem.*, 2019, **91**, 11397-11402.
- [4] Q. W. Song, C. Z. Chen, W. H. Yu, L. X. Yang, K. F. Zhang, J. Zheng, X. Y. Du and H. K. Chen, *RSC Adv.*, 2019, **9**, 25976-25980.
- [5] P. Rana, L. Panda, N. Murmu, B. P. Bag and S. N. Sahu, *Org. Biomol. Chem.*, 2020, **18**, 5963-5971.