

Supporting Information

Design of Fluorescein-Ferrocene Derivatives as HOCl -triggered Turn-on Fluorescent Probe and Anticancer Prodrug

Rui-qi Wang^a, Tong Zhou^a, Aimin Li^b, Jian Qu^{c,*}, Xin Zhang^c, Xiang-feng Zhu^a, Su
Jing^{a,*}

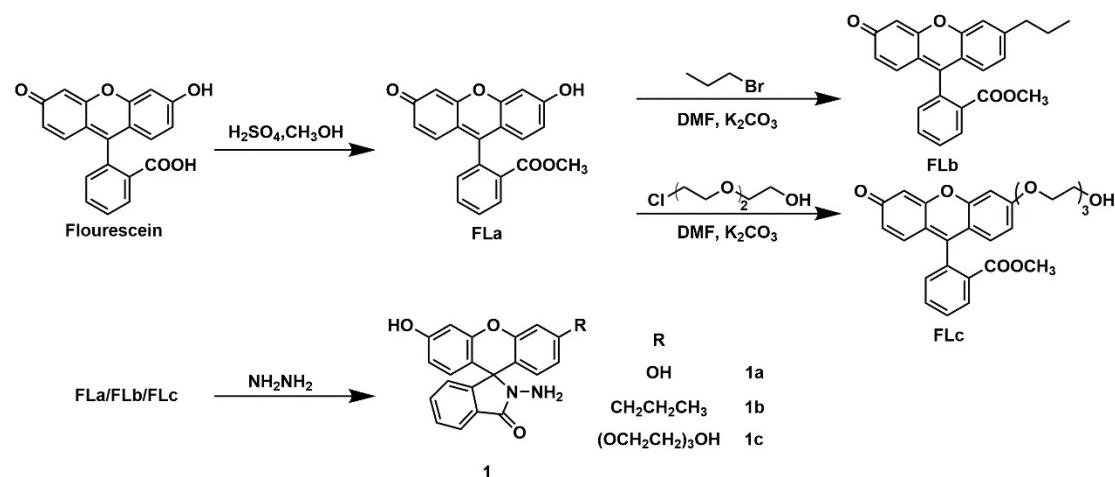
^a School of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing
211816, China. E-mail: sjing@njtech.edu.cn

^b State Key Laboratory of Pollution Control and Resource Reuse, School of the
Environment, Nanjing University, Nanjing 210023, China.

^c School of Materials Science and Engineering, Yancheng Institute of Technology,
Yancheng 224051, China, E-mail: iamqujian@ycit.edu.cn

Synthesis of precursor 1

The precursors of fluorescein derivative 1 were obtained referring to the previous reports.^{1,2} Scheme S1 illustrates the synthetic route of precursors 1. The details of synthesis are as follows.



Scheme S1. Synthetic route of precursors 1.

Compound FLa Typically, fluorescein (3.32 g, 0.01 mol) was dissolved in 100 mL of methanol and concentrated sulfuric acid (1.0 mL) was added dropwise to the solution and refluxed for 8 h. After cooling, excess methanol was removed under reduced pressure and excess water was added to the residue. The red solid **1a** was washed with water several times and dried in vacuum, obtained in 91% yield.

Compound FLb Bromopropane (0.25 g, 2.0 mmol) or 2-[2-(2-chloroethoxy)ethoxy]ethanol (0.34 g, 2.0 mmol), K₂CO₃ (0.27 g, 2.0 mmol) and **compound 1a** (0.35 g, 1.0 mmol) were added into 15 mL DMF and stirred at 120 °C for 12 h. Then the solvent was removed under reduced pressure and the product was purified through a silica gel column eluted with AcOEt/ CH₃OH (v/v = 30:1) or AcOEt/ CH₃OH (v/v = 20:1), obtained in 88% yield.

Compound FLc 2-[2-(2-Chloroethoxy)ethoxy]ethanol (0.34 g, 2.0 mmol), K₂CO₃ (0.27 g, 2.0 mmol) and **compound 1a** (0.35 g, 1.0 mmol) were added into 15 mL DMF and stirred at 120 °C for 12 h. Then the solvent was removed under reduced pressure and the product was purified through a silica gel column eluted with AcOEt/ CH₃OH

($v/v = 30:1$) or AcOEt/ CH₃OH ($v/v = 20:1$), obtained in 85% yield.

Compound 1 Compound **FLa/FLb/FLc** (0.40 g) and hydrazine hydrate (0.24 g, 4.8 mmol) were added to 5.0 mL methanol, refluxed and stirred for 6 h. The solvent was removed under reduced pressure and the product was washed three times with water. The solid was obtained in 89% (**1a**), 84% (**1b**) and 80% (**1c**) yield accordingly.

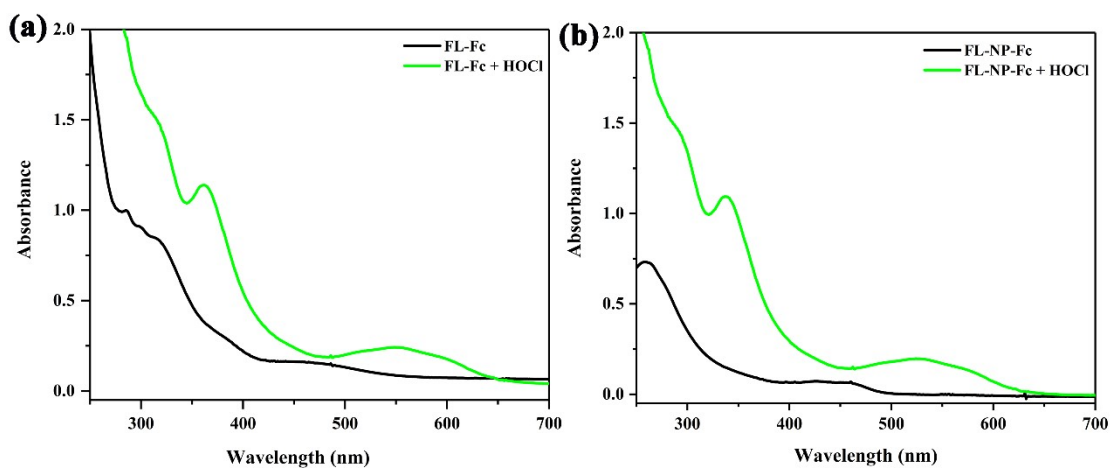


Figure S1. UV-vis absorption spectra of (a) **FL-NP-Fc** with or without HOCl in DMSO/PBS buffer solution (1/10, v/v , 5.0 μ M, pH = 7.4) and (b) **FL-Fc** with or without HOCl in DMSO/PBS buffer solution (1/10, v/v , 5.0 μ M, pH = 7.4)

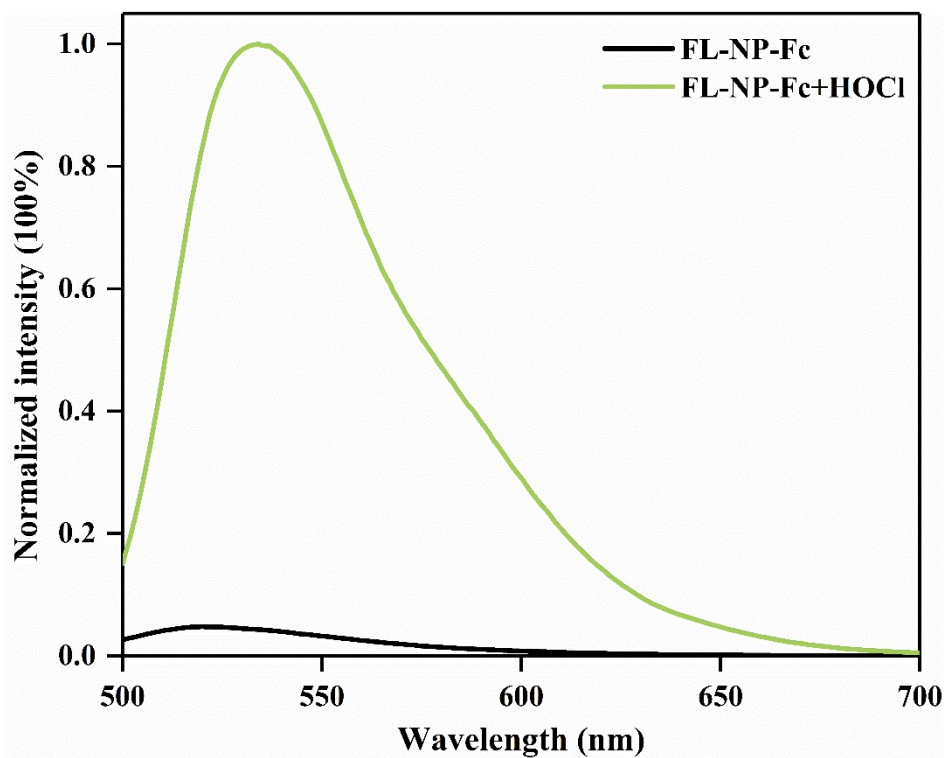


Figure S2. Fluorescence emission spectra of FL-NP-Fc with or without HOCl in DMSO/PBS buffer solution (1/10, v/v, 5.0 μ M, pH = 7.4) (λ_{ex} = 488 nm).

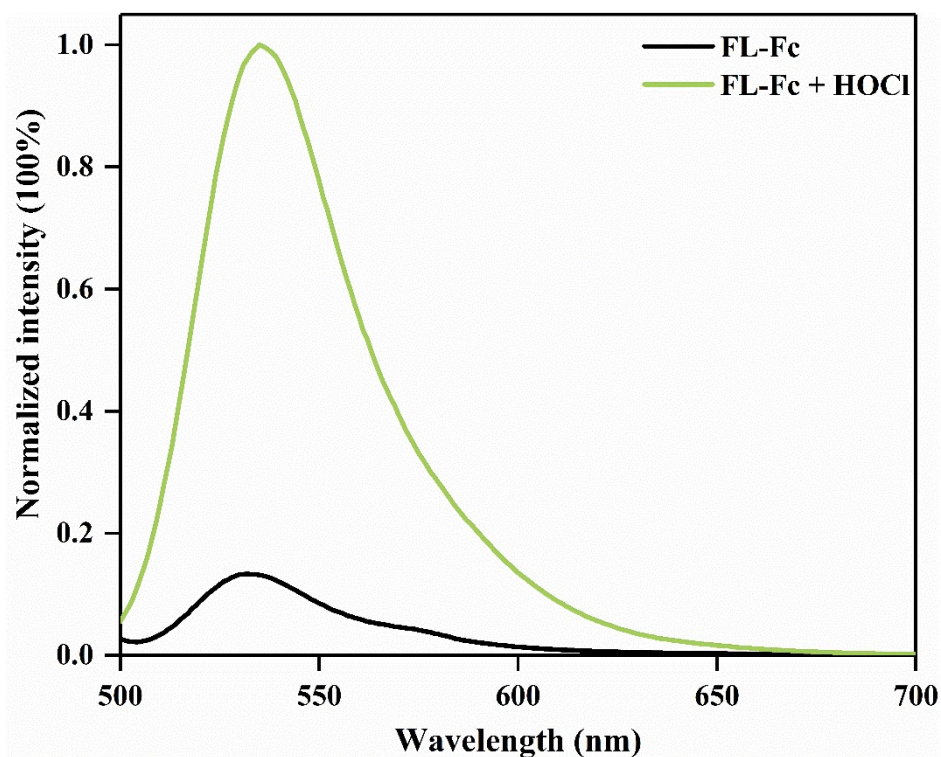


Figure S3. Fluorescence emission spectra of FL-Fc with or without HOCl in

DMSO/PBS buffer solution (1/10, v/v, 5.0 μ M, pH = 7.4) (λ_{ex} = 488 nm).

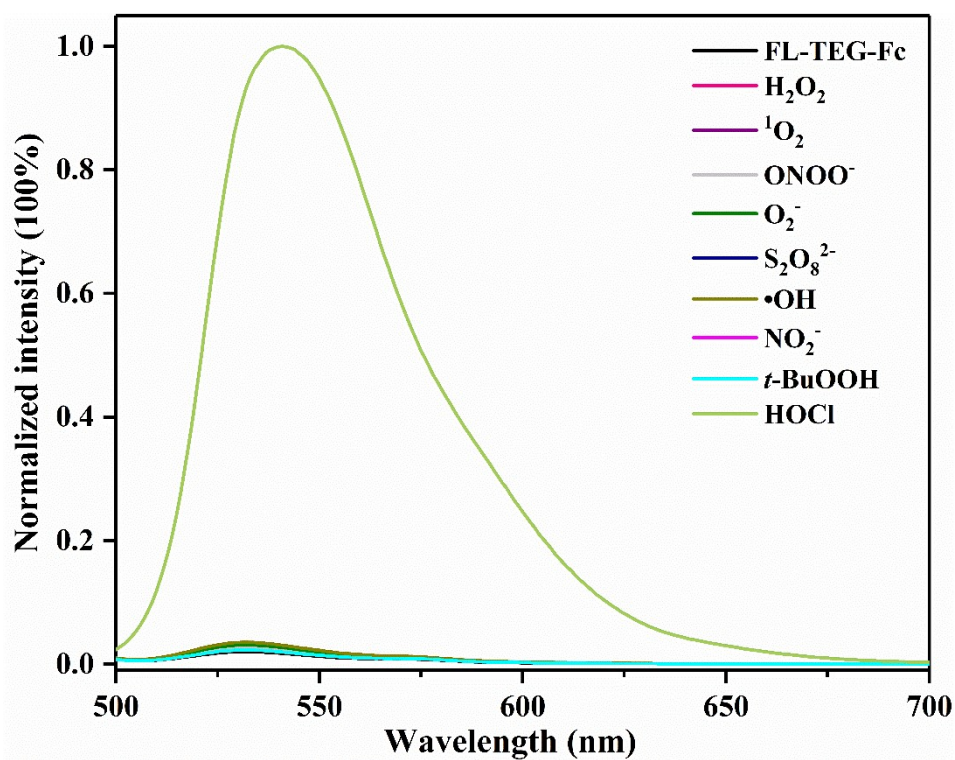


Figure S4. Fluorescence emission changes of **FL-TEG-Fc** against ROS/RNS in DMSO/PBS buffer solution (1/100, v/v, 0.25 mM, pH = 7.4) (λ_{ex} = 488 nm).

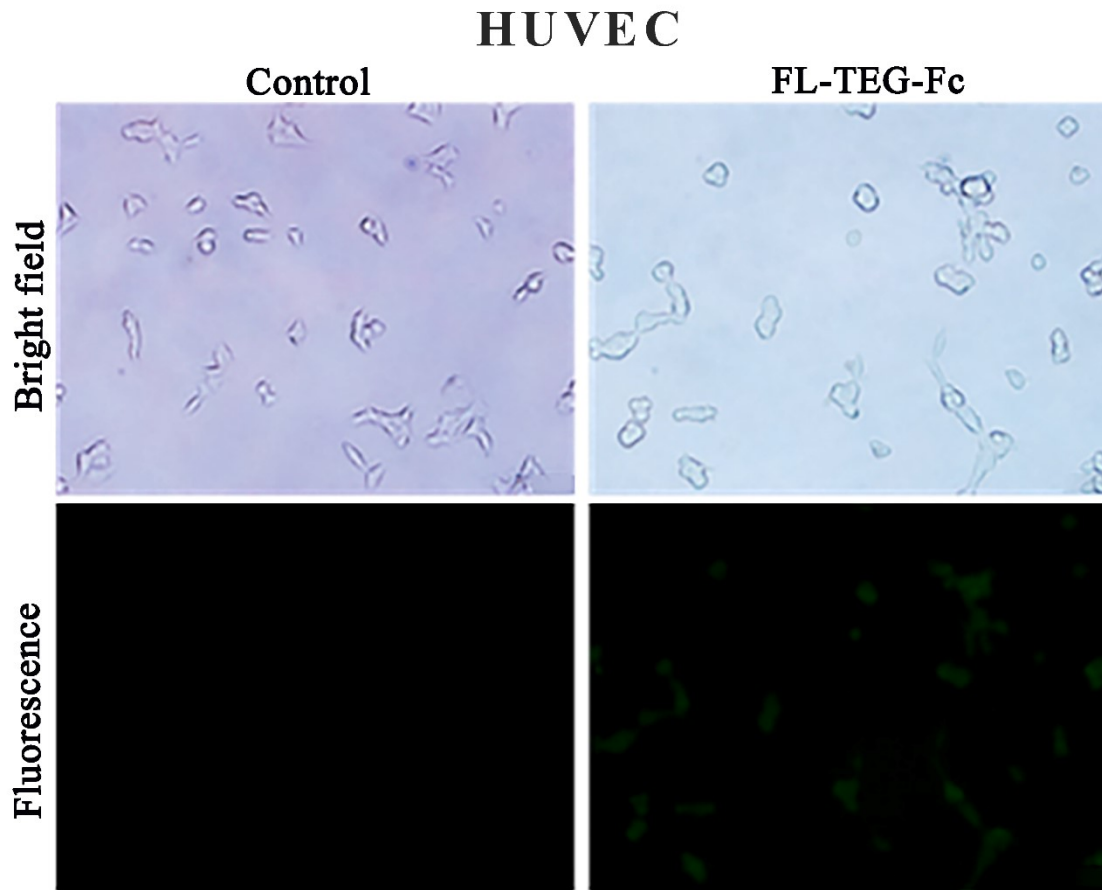


Figure S5. Fluorescence images of HUVEC cells treated with 100 μ M of FL-TEG-Fc in DMEM for 1 h. Images were obtained by fluorescence microscopy.

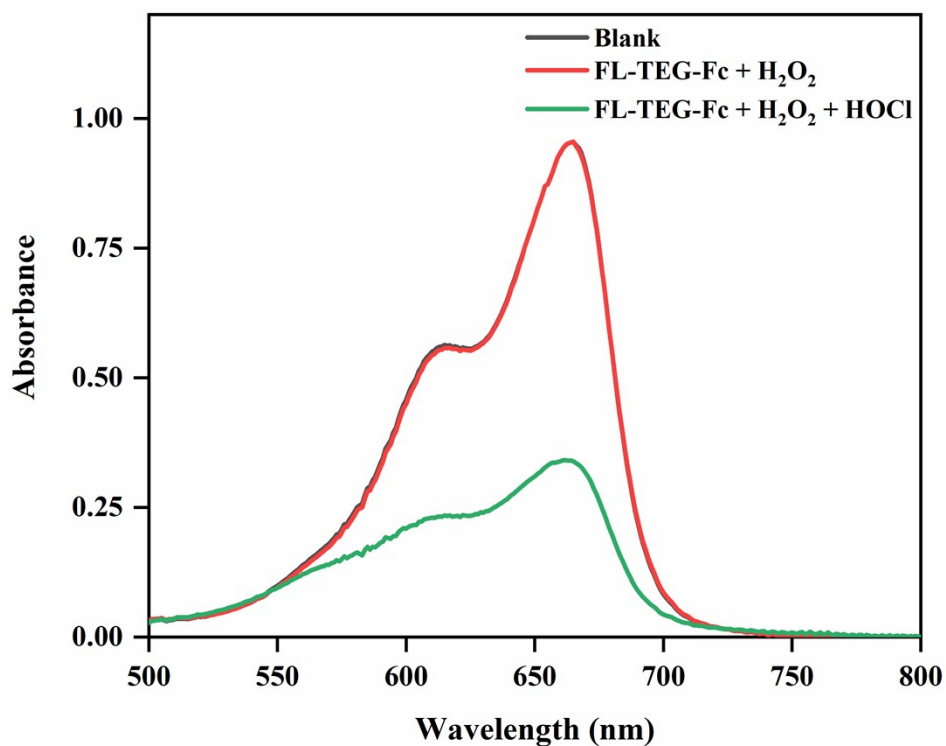


Figure S6. UV-vis spectra of MB treated by different reagents.

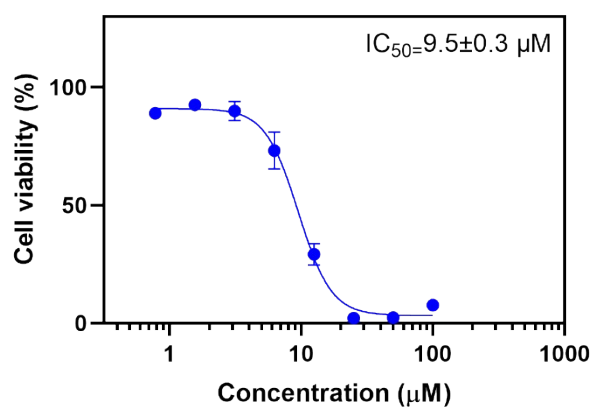


Figure S7. Anticancer activities of FL-TEG-Fc in AGS cells.

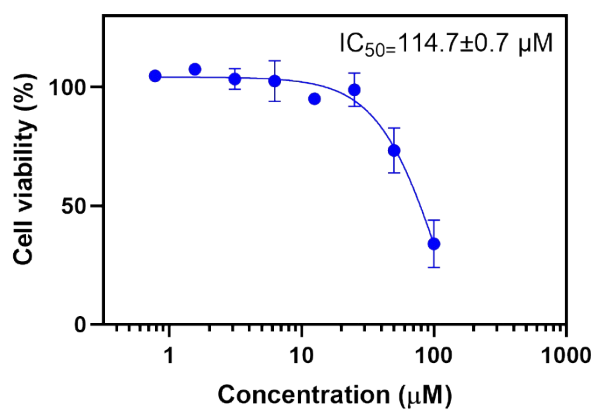


Figure S8. Anticancer activities of FL-TEG-Fc in HUVEC cells.

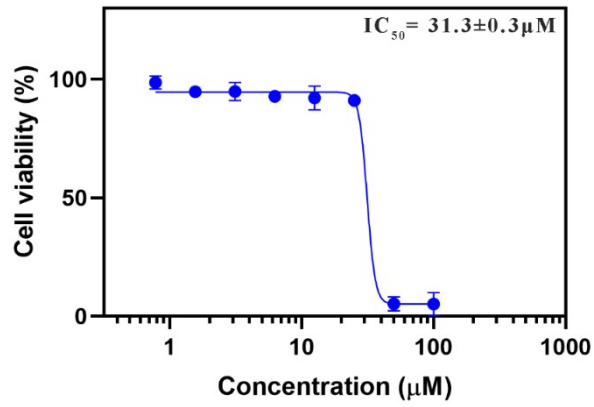


Figure S9. Anticancer activities of **FL-NP-Fc** in AGS cells.

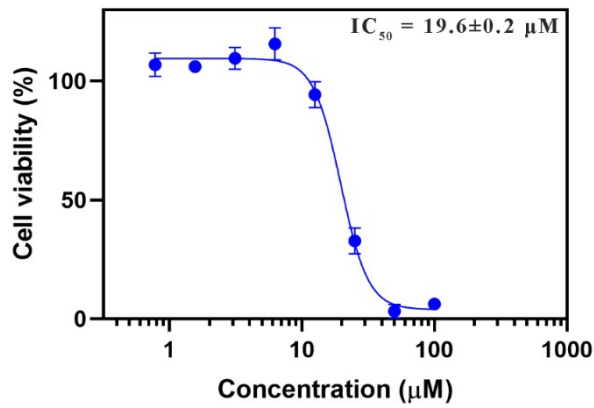


Figure S10. Anticancer activities of **FL-Fc** in AGS cells.

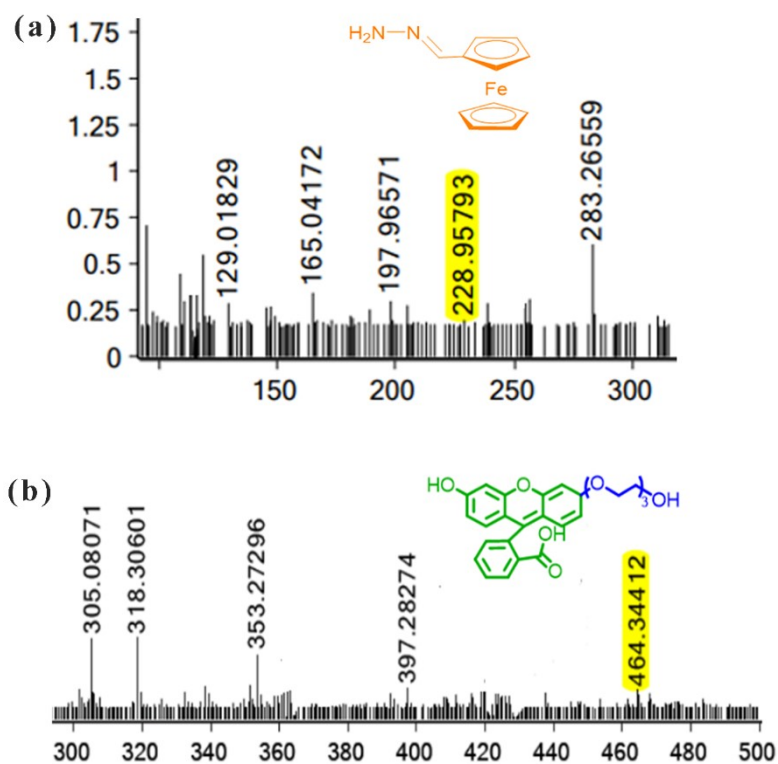
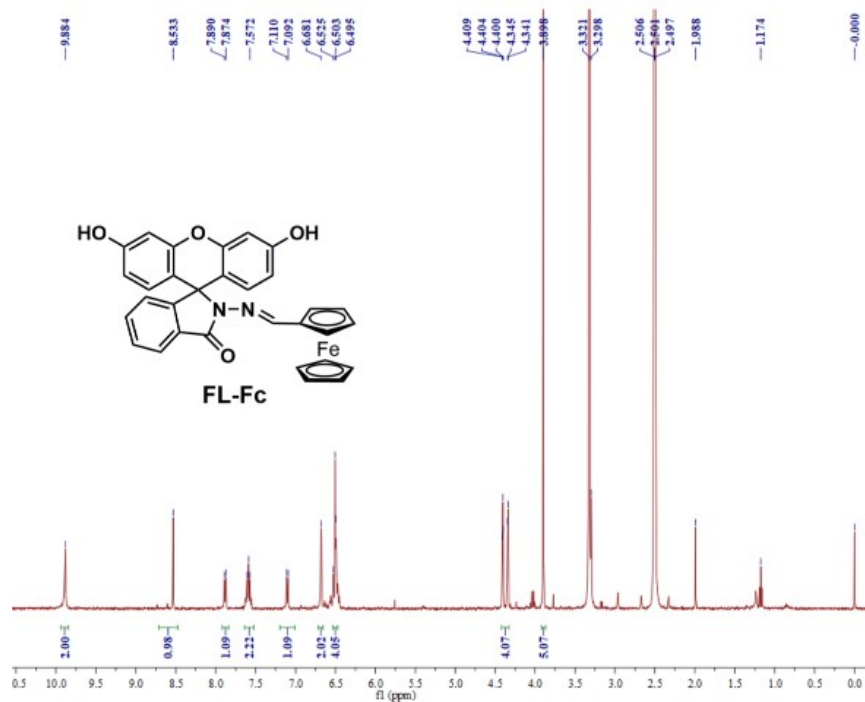


Figure S11. Mass spectrometry spectra of dissociative products of FL-TEG-Fc with HOCl.



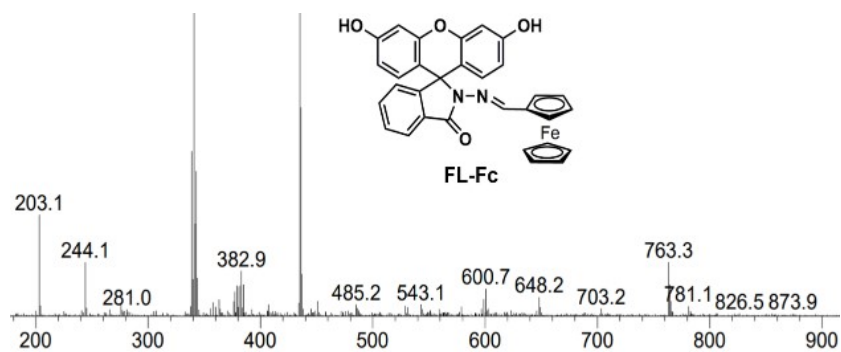


Figure S12. ¹H NMR and HRMS spectra for compound FL-Fc.

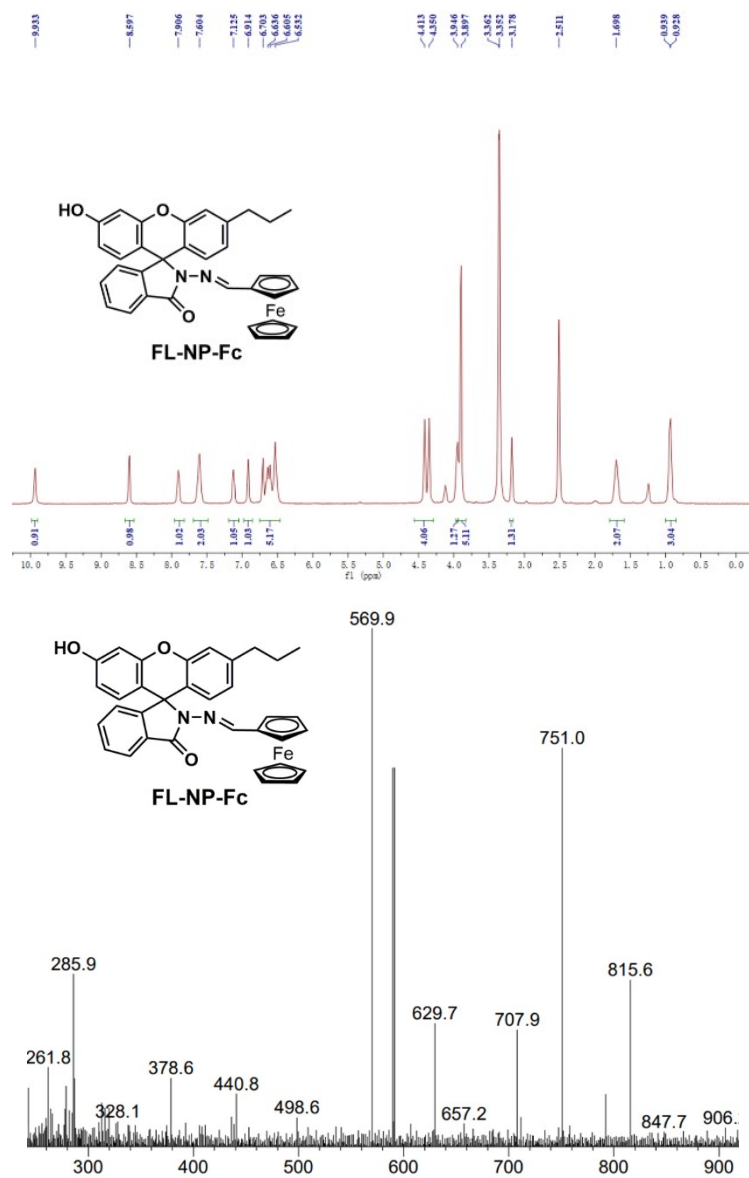


Figure S13. ¹H NMR and MS spectra for compound FL-NP-Fc.

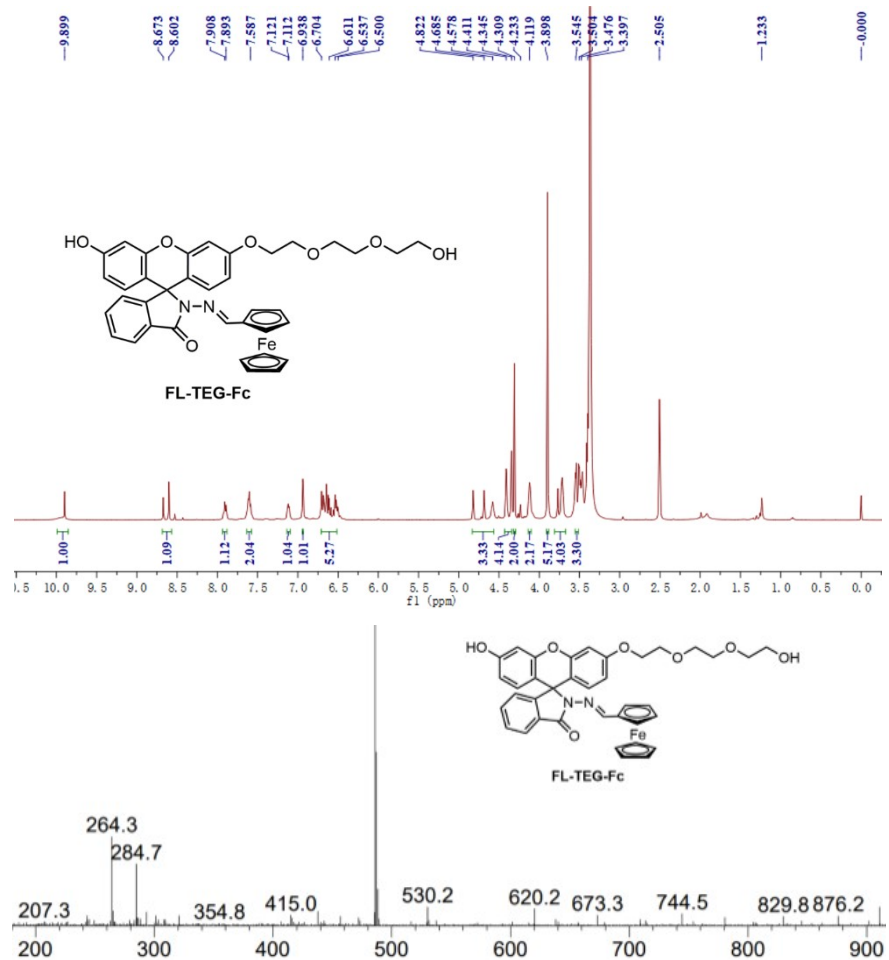
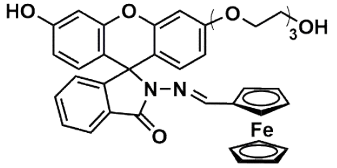
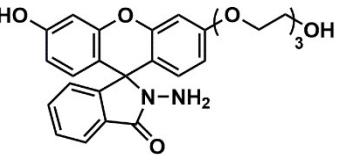
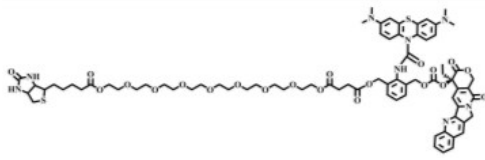
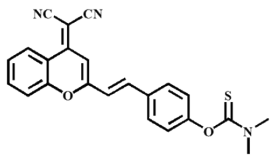
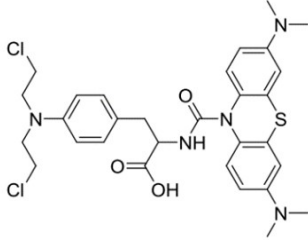
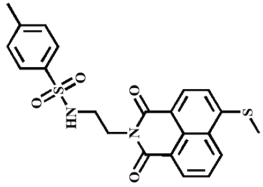
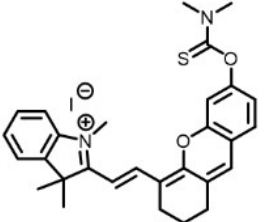


Figure S14. ¹H NMR and MS spectra for compound **FL-TEG-Fc**.

Table S1 Comparison of FL-TEG-Fc with other representative HOCl probes.

Entry	Probes	$\lambda_{\text{ex}}/\lambda_{\text{em}}$ (nm)	Response Time	Detection Limit (μM)	Applications	Reference
1	 <p style="text-align: center;">FL-TEG-Fc</p>	488/523	60 s	6.5	Sensing, imaging and anticancer prodrug	This work
2		488/520	2 min	-	Sensing and imaging	[1]
3		620/686	3 min	--	Sensing, imaging and anticancer prodrug	[3]

4		585/730	7 s	0.11	Sensing and imaging	[4]
5		450/520	40 s	0.04	Sensing and imaging	[5]
6		410/490	400 s	2.16	Sensing and imaging	[6]
7		556/627	5 s	0.007	Sensing and imaging	[7]
8		383/520	80 s	0.012	Sensing and imaging	[8]

9		545/685	5 min	0.164	Sensing and imaging	[9]
10		620/686	60 s	--	Sensing, imaging and anticancer prodrug	[10]
11		365/509	150 s	0.12	Sensing and imaging	[11]
12		685/725	5 s	0.131	Sensing and imaging	[12]

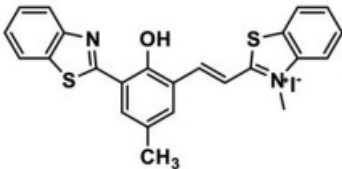
13		450/552	5 min	0.13	Sensing and imaging	[13]
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Table S2. Cytotoxicities of ferrocene-fluorescein derivatives and fluorescein precursors in AGS and HUVEC cells for 72 h.

Compounds	IC ₅₀ (μM)	
	AGS	HUVEC
FL	>100	>100
FL-Fc	19.6 ± 0.2	>100
FL-NP	>100	>100
FL-NP-Fc	31.3 ± 0.3	>100
FL-TEG	>100	>100
FL-TEG-Fc	9.5 ± 0.3	>100

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