Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

## Ultra-sensitive fluorescent probes for hypochlorite acid detection and exogenous/endogenous imaging of living cells

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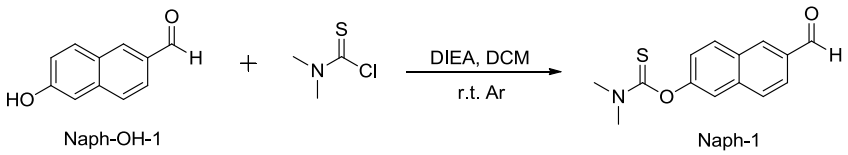
# 1. Experimental Section

## 1.1 Materials and chemicals

All chemicals used for synthesis were purchased from commercial suppliers and applied directly without purification. 6-hydroxy-2-naphthaldehyde (Naph-Oh-1), 1-(6-methoxynaphthalen-2-yl)ethenone (Naph-$OCH_3$-2) and dimethylcarbamothioic chloride were purchased from J&K Chemical. Anhydrous dichloromethane (DCM) and methanol were purchased from Adamas-beta. Dulbecco's modified Eagle's medium (DMEM), PBS, fetal bovine serum (FBS), trypsin-EDTA and penicillin/streptomycin were purchased from GBICO. All reactions that utilize air- or moisture sensitive reagents were performed in dried glassware under dry Ar. Milli-Q water was used in all experiments. All spectroscopic measurements were performed in phosphate-buffered saline (PBS, 10 mM, pH 7.4). The progress of the reaction was monitored by thin-layer chromatography (TLC; Merck 60F-254). Merck silica gel 60 (70−200 mesh) was used for general column chromatography purification purpose. $^{1}H$ NMR and $^{13}C$ NMR spectra were taken on a Bruker 400 MHz NMR spectrometer. For $^{1}H$ NMR, coupling constants (J) are reported in Hertz (Hz), and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), and bs (broad singlet). Mass spectra were obtained using AB Sciex 3200 QTrap ESI-MS system or Bruker ultrafleXtreme MALDI-TOF/TOF. UV absorption spectra were obtained on Shimadzu 1700 UV/vis Spectrometer. Fluorescence spectra were acquired with a FluoroMax-4 fluorescence photometer. Fluorescence images were captured using a Leica SPE Confocal Scanning Microscope.

## 1.2 Synthesis and characterization

### 1.2.1 Synthesis of Naph-1

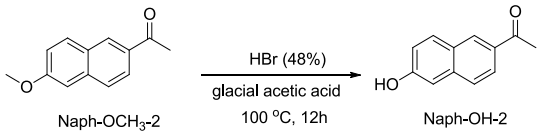



Naph-OH-1 Naph-1

Naph-OH-1 (2 mmol, 348 mg) and N,N-diisopropylethylamine (DIEA, 4 mmol, 697 μL) were dissolved in dry DCM (10 mL) under argon atmosphere. Then,

dimethylcarbamothioic chloride (4 mmol, 488 mg) was added into the reaction solvent. The mixture was stirred under room temperature overnight. After the reaction complete by TLC monitor, another portion of DCM (20 mL) was added and washed with water and brine. The organic layer was separated and dried with $Na_2SO_4$. The crude product was purified by silica gel column chromatography with pure DCM to obtain Naph-1 as a white colour powder (321 mg, 62% yield); $^1$H NMR (400 MHz, $CDCl_3$): δ = 10.15 (s, 1H), 8.34 (s, 1H), 8.02 (d, *J* = 8 Hz, 1H), 7.96 (dd, *J* = 8 Hz, 1.2 Hz, 1H), 7.89 (d, *J* = 8 Hz, 1H), 7.56 (d, *J* = 2 Hz, 1H), 7.37 (dd, *J* = 8 Hz, 2 Hz, 1H), 3.49 (s, 3H), 3.42 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 192.06, 187.23, 154.05, 136.99, 134.25, 134.02, 130.67, 130.59, 128.86, 123.99, 123.46, 119.98, 43.35, 38.93; ESI-MS: calcd. for $C_{14}H_{14}NO_2S$ $[M+H]^+$ 260.067, found 260.00.

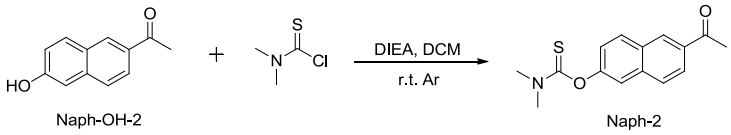
### 1.2.2 Synthesis of Naph-OH-2




Naph-OH-2 was synthesized following our reported method.[1]

[1] H. Zhang, P. Xiao, Y. T. Wong, W. Shen, M. Chhabra, R. Peltier, Y. Jiang, Y. He, J. He, Y. Tan, Y. Xie, D. Ho, Y.-W. Lam, J. Sun, H. Sun, Construction of an alkaline phosphatase-specific two-photon probe and its imaging application in living cells and tissues, Biomaterials, 2017, 140, 220–229

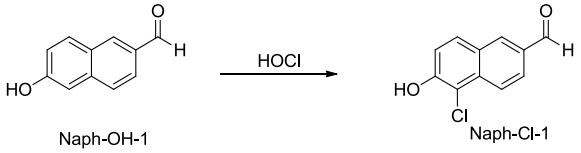
### 1.2.3 Synthesis of Naph-2




Naph-OH-2 (0.5 mmol, 93 mg) and N,N-diisopropylethylamine (DIEA, 1 mmol, 174 μL) were dissolved in dry DCM (10 mL) under argon atmosphere. Then, dimethylcarbamothioic chloride (1 mmol, 122 mg) was added into the reaction solvent. The mixture was stirred under room temperature overnight. After the reaction complete by TLC monitor, another portion of DCM (20 mL) was added and washed with water and brine. The organic layer was separated and dried with $Na_2SO_4$. The crude product was purified by silica gel column chromatography with DCM/Hexane =

5/1 to obtain Naph-2 as a white colour powder (72 mg, 53% yield); $^{1}$H NMR (400 MHz, $CDCl_3$): δ = 8.44 (s, 1H), 8.02 (d, *J* = 8 Hz, 1H), 7.95 (d, *J* = 8 Hz, 1H), 7.83 (d, *J* = 8 Hz, 1H), 7.52 (s, 1H), 7.32 (d, *J* = 8 Hz, 1H), 3.47 (s, 3H), 3.39 (s, 3H), 2.70 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 197.90, 187.46, 153.66, 136.14, 134.51, 130.65, 130.06, 128.27, 124.64, 123.70, 119.67, 43.38, 38.93, 26.74; ESI-MS: calcd. for $C_{15}H_{16}NO_2S$ $[M+H]^+$ 274.082, found 274.005.

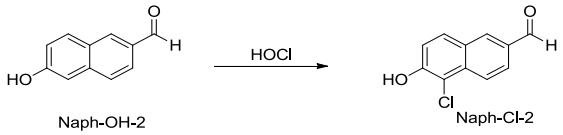
### 1.2.4 Synthesis of Naph-Cl-1




Naph-OH-1 Naph-Cl-1

Naph-OH-1 (0.23 mmol, 40 mg) was dissolved in methanol (5 mL). Then, NaOCl (300 µL) was added into the reaction solvent at room temperature. After 5 min later, the solvent was removed under vacuum. The solid product was purified by column chromatography using DCM/Hexane = 20:1 to obtain Naph-Cl-1 as a light yellow solid (40.7 mg, 86% yield); $^{1}$H NMR (400 MHz, *d*$^{6}$-DMSO): 11.06 (s, 1H), 10.10 (s, 1H), 8.52 (s ,1H), 8.15 (d, *J* = 8 Hz, 1H), 8.04 (d, *J* = 8 Hz, 1H), 7.97 (dd, *J* = 8 Hz, 1.2 Hz, 1H), 7.42 (d, *J* = 8 Hz, 1H); ESI-MS: calcd. for $C_{11}H_7ClO_2$ $[M-H]^-$ 205.0, found 205.1.

### 1.2.5 Synthesis of Naph-Cl-2




Naph-OH-2 Naph-Cl-2

Naph-OH-2 (0.27 mmol, 50 mg) was dissolved in methanol (5 mL). Then, NaOCl (400 µL) was added into the reaction solvent at room temperature. After 5 min later, the solvent was removed under vacuum. The solid product was purified by column chromatography using DCM/Hexane = 10:1 to obtain Naph-Cl-2 as a white solid (47.5 mg, 80% yield); $^{1}$H NMR (400 MHz, *d*$^{6}$-DMSO): 10.91 (s, 1H), 8.62 (s ,1H), 8.08 (d, *J* = 8 Hz, 1H), 8.04 (dd, *J* = 8 Hz, 1.2 Hz, 1H), 8.0- (d, *J* = 8 Hz, 1H), 7.39 (d, *J* = 8 Hz, 1H), 2.67 (s, 3H); ESI-MS: calcd. for $C_{12}H_9ClO_2$ $[M-H]^-$ 219.0, found 219.2.

**1.3 Determination of detection limit**

Detection limit or limit of detection was calculated based on the fluorescence titration curve of Naph-1 or Naph-2 in the presence of HOCl and mean of the blank. In detailed, the fluorescence intensity of Naph-1 or Naph-2 was measured and the standard deviation of blank measurements was achieved and marked as σ using the following equation. σ is the standard deviation of the blank measurements; n is the tested number of blank measure (n= 11); $\bar{x}$ is the mean of the blank measures; $X_i$ is the values of the blank measures.

$$\sigma = \sqrt{\frac{\Sigma(\bar{x}-x_i)^2}{n-1}}$$

In this manuscript, we use the following equation for the calculation of detection limit:

Detection limit = 3σ/s

s is the slope of fluorescence intensity versus HOCl concentrations.

**1.4 Absorption and fluorescence measurement**

Naph-1 or NaPh-2 was dissolved in an appropriate amount of DMSO to obtain 5 mM stock solution. NaOCl and other biological analytes were prepared as 1 mM or 10 mM stock solutions in PBS buffer. All the measurement was taken under room temperature. Firstly, Naph-1 or Naph-2 was diluted in PBS buffer (10 mM, pH 7.4) to afford a final concentration of 5 μM to a 10-mm quartz cuvette. Then, different concentration of NaOCl or other analytes was added into the quartz cuvette for 1 min incubation. After that, the absorption or fluorescence was measured using Shimadzu 1700 UV/vis Spectrometer and FluoroMax-4 fluorescence photometer respectively. Specially, for the preparation of reactive oxygen species, such as HO•, $O_2^{\bullet-}$, •O$^t$Bu, the protocol from Yang's group was followed. [2]

[2] Jun Jacob Hu, Nai-Kei Wong, Sen Ye, Xingmiao Chen, Ming-Yang Lu, Angela Qian Zhao, Yuhan Guo, Alvin Chun-Hang Ma, Anskar Yu-Hung Leung, Jiangang Shen, and Dan Yang, *J. Am. Chem. Soc.*, **2015**, 137, 6837–6843.

**1.5 MTS assay**

HeLa cells were seeded into 96-well plates at a density of $5\times10^4$ cells per well in

200 μL of DMEM supplemented with 10% heat-inactivated FBS (fetal bovine serum) and 1% penicillin/streptomycin. Cells were allowed to grow overnight under an atmosphere of 5% $CO_2$ at 37 $^oC$. The culture media was replaced by the new fresh medium with the various concentration of Naph-1 or Naph-2 (0, 5, 10, 20, 50 μM). The cells were incubated at the same condition for 24 h. Subsequently, 20 μL of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS, 2 mg/mL) was added and the cells were further cultured for another 2 h. Finally, measure the absorbance of treated cells using the plate reader at OD=490nm.

**1.6 Cell culture**

HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin). Approximately $10^5$ cells were seeded in a confocal dish (35 mm). 4T1 cells and RAW 264.7 cells maintained in the same manner as HeLa cells.

**1.7 Confocal fluorescence imaging**

For HeLa cells and 4T1 cells imaging, the cells were treated with Naph-1 or Naph-3 (10 μM) at 37 $^oC$ for 30 min and then incubated with NaOCl (50 μM) for another 30 min. Cells treated with Naph-1 or Naph-2 (10 μM) alone were used as a control.

For endogenous HOCl test, RAW 264.7 cells were incubated with lipopolysaccharides (LPS, 5 μg/mL) and phorbol myristate acetate (PMA, 5 μg/mL) for 12 h, then Naph-1 or Naph-2 (10 μM) was added into the medium for 1 h incubation. For inhibition experiment, RAW 264.7 cells were incubated with lipopolysaccharides (LPS, 5 μg/mL), phorbol myristate acetate (PMA, 5 μg/mL) and 4-aminobenzoic acid hydrazide (ABH, 400 μM) for 12 h, then Naph-1 or Naph-2 (10 μM) was added into the medium for 1 h incubation. Fluorescence images were taken using a Leica TCS SPE Confocal Scanning Microscope. Ex: 405 nm, Em: 505-555 nm for Naph-1, 485-535 nm for Naph-2.

# 2 Supplemental tables and figures

Table S1. Properties of representative fluorescent HOCl probes

| probe | λex/ nm | λem /nm | FEF[a] | Detection limit/nM[b] | Reaction time[c] | Solution[d] | Imaging application[e] |
|---|---|---|---|---|---|---|---|
| $[Ru(bpy)_2(AN\text{-}bpy)](PF_6)_2$ Inorg. Chem. 2013, 52, 10325 | 456 | 612 | 110 fold | 33 | within 10 min | borate buffer (pH 7.4) | Endogenous / Porcine neutrophils |
| Cou-Rho-HOCl Chem. Eur. J. 2012, 18, 2700 | 410 | 473 | 594 | 52 | Within minutes | PBS/DMF (1:1, pH 7.4) | Endogenous / RAW 264.7 cells |
| HKOCl-2b Org. Lett. 2014, 16, 3544 | 523 | 545 | 908 | 18 | within 15 min | PBS (pH 7.4) | Endogenous / macrophages |
| HKOCl-1 Org. Lett. 2008, 10, 2171 | ~ | 541 | 1079 | ~ | ~ | PBS (pH 7.5) | Endogenous / RAW 264.7 macrophages |
| Bodipy-OX Chem. Commun. 2013, 49, 7836 | 502 | 529 | 48 | 500 | within seconds | PBS/DMF (4:1, pH 7.2) | Exogenous / MCF10A cell |
| BClO J. Am. Chem. Soc. 2014, 136, 12820 | 500 | 505 | 100 | 0.56 | within 1 s | PBS/EtOH (1:9, pH 7.4) | Endogenous / MCF-7 cells |
| SeCy7 Chem. Commun. 2014, 50, 1018 | ~ | 786 | 19.4 | 310 | Within dozens of seconds | PBS (pH 7.4) | Endogenous / Model mouse |
| CM1 Org. Lett. 2013, 15, 2002 | 405 | 480 | 63 | 10 | within seconds | PBS (pH 7.4) | Endogenous / RAW264.7 cells |
| Lyso-NI-Se J. Photoch. Photobio. A , 2015, 299, 1 | 450 | 540 | 22 | 18.5 | Within seconds | $CH_3CN/H_2O$ (1:1, pH 5.0) | Exogenous / lysosome of RAW 264.7 cell |
| HCH Sens. Actuators B Chem., 2016, 222, 483 | 860 | 546 | 14.1 | 580 | 20 minutes | PBS (pH 7.4) | Exogenous/ mitochondria of CHO cells |
| PZ–Py Chem. Commun. 2015, 51, 1442 | 400 | 562 | 40.5 | 17.9 | Within seconds | PBS (pH 7.3) | Endogenous / mitochondria of RAW 264.7 cells and nude mice |
| Rh-Py Chem. Commun. 2014, 50, 8640 | 544 | 577 | 380 | 24 | Within seconds | PBS (pH 7.4) | Exogenous/ mitochondria of HeLa cells and nude mice |
| HQ Dalton Trans. 2015, 44, 6613 | 740 | 495 | 23.5 | 310 | 10 minutes | PBS/DMSO (1:9, pH 7.4) | Exogenous / HeLa cell |
| NDMTC Anal. Chem. 2016, 88, 12532 | 800 | 547 | ~ | 0.0076 | 2 minutes | PBS/ethanol (1:1, pH 7.4) | Endogenous / RAW 264.7, cancerous breast tissue of mice |
| MITO-TP J. Am. Chem. Soc. 2015, 137, 5930 | 375 | 500 | 634 | 17.2 | Within seconds | PBS (PH 7.4) | Endogenous /mitochondria of RAW 264.7 cells and inflamed tissues |
| LYSO-TP J. Am. Chem. Soc. 2015, 137, 5930 | 375 | 500 | 610 | 19.6 | Within seconds | PBS (PH 5.0) | Endogenous / lysosome of RAW 264.7 cells and inflamed tissues |
| PNIS Anal. Chem. 2016, 88, 6615 | 700 | 447 | ~ | 210 | 3 minutes | PBS (PH 7.4) | Exogenous /Mitochondria of HeLa cells, astrocytes, and HepG2 cells; Endogenous /RAW 264.7 cells |
| PIS Angew. Chem. Int. Ed. 2015, 54, 4890 | 800 | 505 | ~ | 71 | ~ | PBS (pH 7.4) | Endogenous / RAW 264.7 cells and rat hippocampal slice |
| NBD-DOP Chem. Commun. 2017, 53, 12349 | 470 | 540 | 23 | 9.7 | Within seconds | PBS (PH 7.4) | Endogenous / HeLa cells |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| STP-HOCl<br>Adv. Funct. Mater.<br>2016, 26, 8128 | 810 | 470/<br>520 | 20.1 | ~ | 1 minute | PBS (pH 7.4) | Exogenous/HeLa cells and liver tissues; Endogenous /RAW 264.7 macrophage cells |
| RTP-HOCl 1<br>Chem. Commun.<br>2017, 53, 10800 | 900 | 598/<br>633 | ~ | 34.8 | within seconds | PBS (pH 7.4) | Endogenous /HeLa cells and mouse brain tissues |
| NCS-BOD-OCH3<br>J. Mater. Chem.B<br>2017, 5, 5854 | 800 | 656/<br>688 | 1.5 | 1150 | 10 minutes | PBS/THF<br>(1/1, pH 7.4) | Exogenous /mitochondria of A357 cells |
| SF-1<br>Chem. Commun.<br>2018, 54, 1849 | 550 | 586 | ~ | 4.3 | within 15 min | PBS/ACN<br>(9/1, v/v) | Endogenous /<br>RAW 264.7 cells |
| FDOCl-1<br>Chem. Sci.,<br>2018, 9, 495 | 620 | 686 | 2068 | 2.62 | within 30 seconds | PBS (pH 7.2) and 0.1% EtOH | Endogenous /<br>RAW 264.7 cells |
| TPP-TCF Analyst, 2018,<br>DOI:10.1039/C8AN00586A | 488 | 660 | 582 | 290 | within several seconds | PBS/DMSO<br>(8/2, pH 7.4) | Exogenous / Hela cells and nude mouse |
| HySOx<br>J. Am. Chem. Soc.<br>2007, 129, 7313 | 555 | 575 | ~ | ~ | within seconds | PBS (pH 7.4) | Endogenous /<br>phagosomes |
| Hypo-SiF<br>J. Am. Chem. Soc.<br>2013, 135, 13365 | 586 | 606 | ~ | ~ | within 80 seconds | PBS (pH 7.4) | No data |
| Naph-2<br>This work | 364 | 510 | 900 | 8.2 | 45 seconds | PBS (pH 7.4) | Exogenous / Hela cells<br>Endogenous / RAW 264.7 cells |
| Naph-1<br>This work | 390 | 530 | 4600 | 2.37 | 25 seconds | PBS (pH 7.4) | Exogenous / Hela cells<br>Endogenous / RAW 264.7 cells |

[a] The fluorescence enhanced factor or emission ratio changes before and after interaction with HOCl; [b] The reported detection limit of corresponding probes; [c] The reaction time for responding to HOCl; [d] The solution and pH used for spectrum measurement; [e] Imaging of endogenous or exogenous HOCl in cells or tissues.

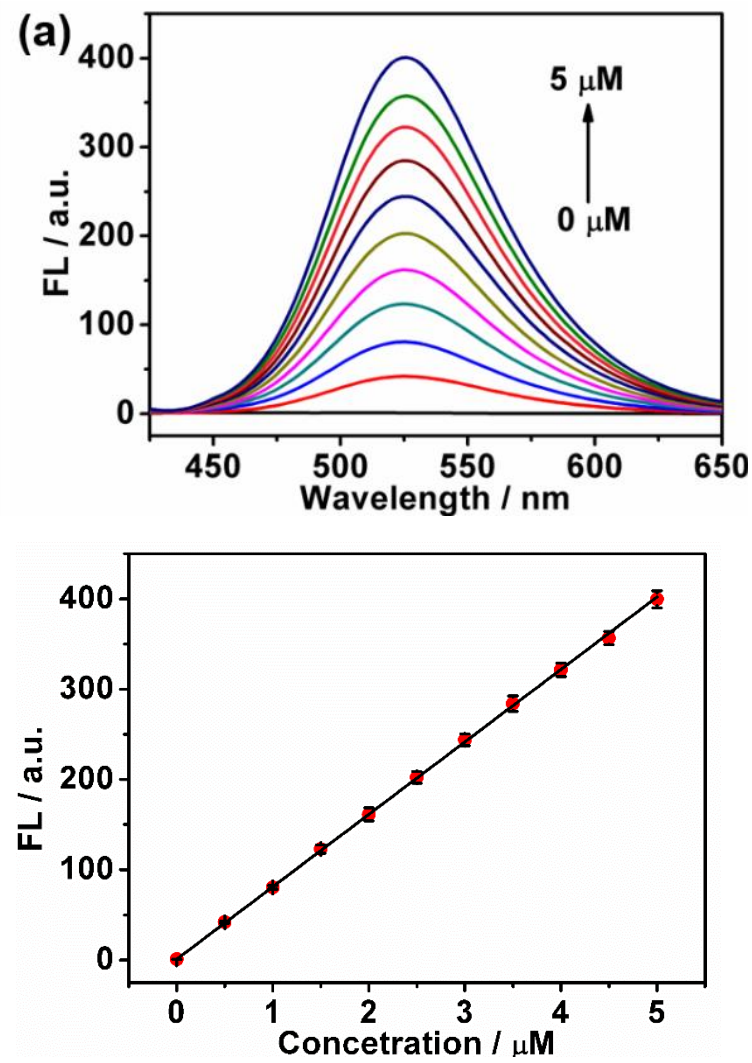



**Fig. S1** (a) Fluorescence spectra of Naph-1 (5 μM) in the presence of a series of HOCl (0-5 μM) in PBS buffer (10 mM, pH 7.4); (b) The linearity between the fluorescent intensity at 530 nm and different concentration of HOCl.

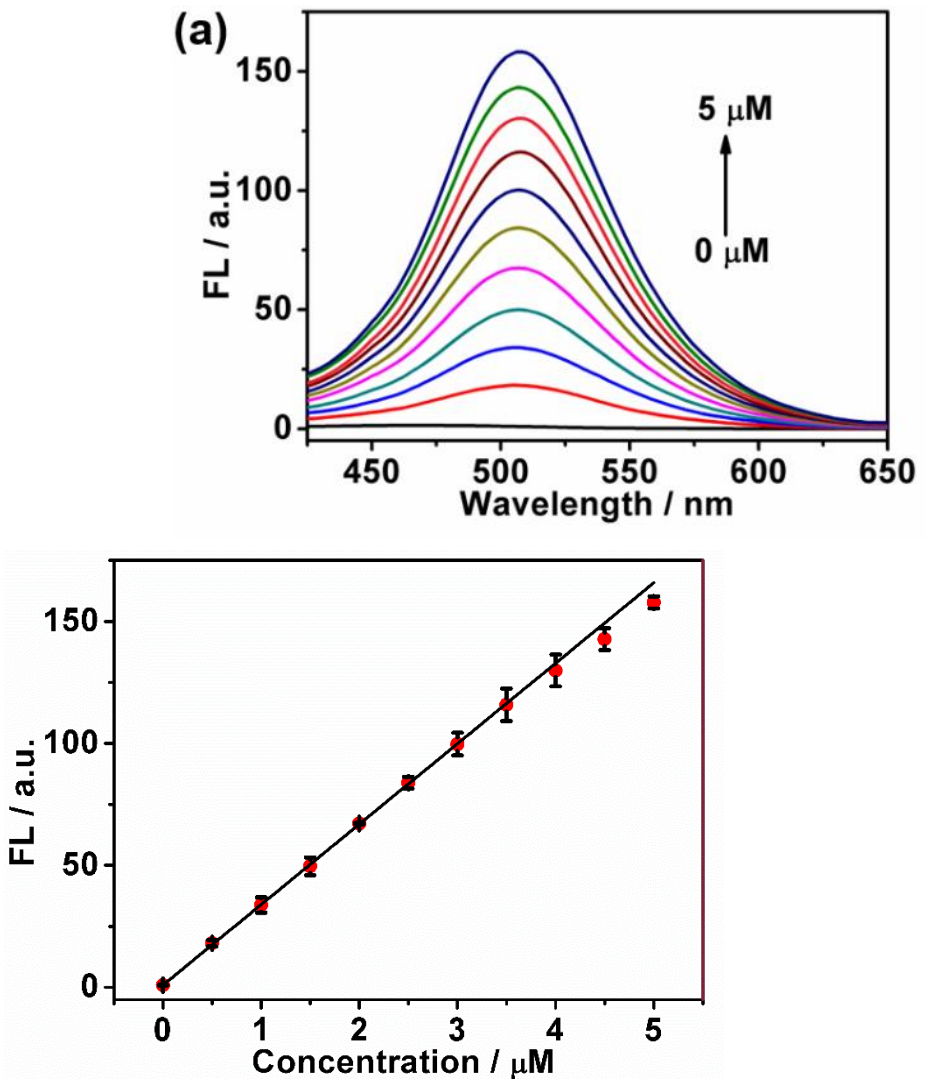



**Fig. S2** (a) Fluorescence spectra of Naph-2 (5 μM) in the presence of a series of HOCl (0-5 μM) in PBS buffer (10 mM, pH 7.4); (b) The linearity between the fluorescent intensity at 510 nm and different concentration of HOCl.

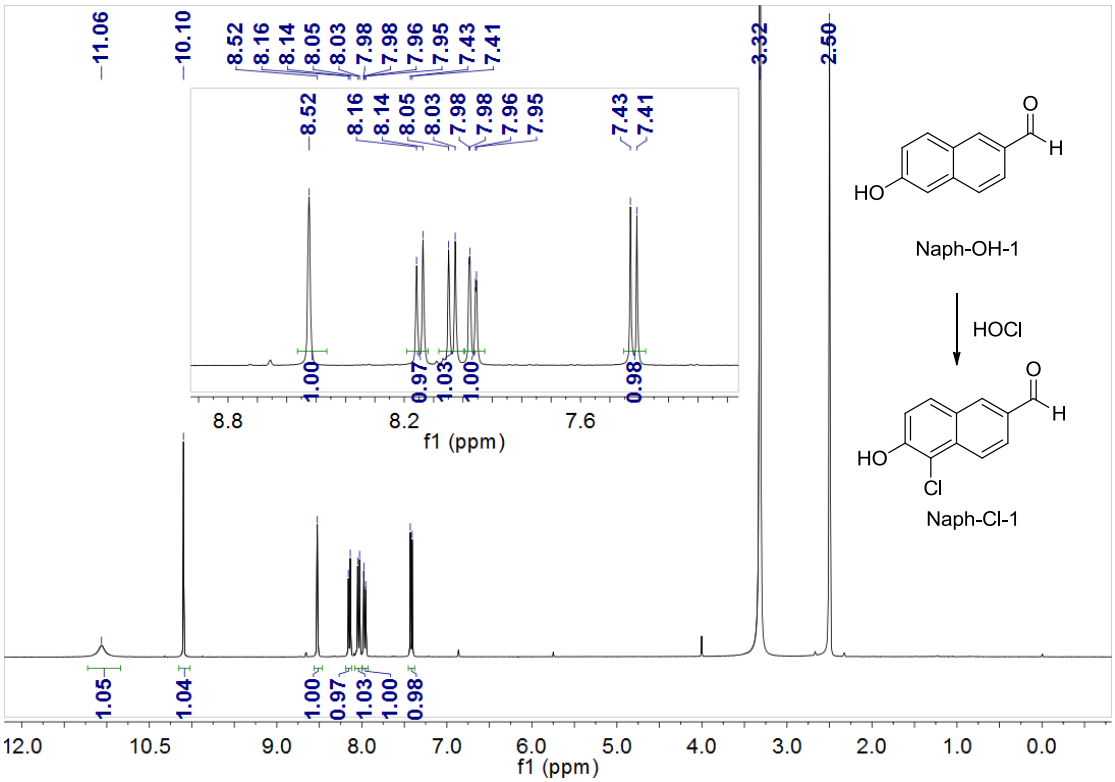



**Fig. S3** $^{1}$H NMR of Naph-Cl-1 from the reaction of Naph-OH-1 and HOCl (*d*$^{6}$-DMSO).

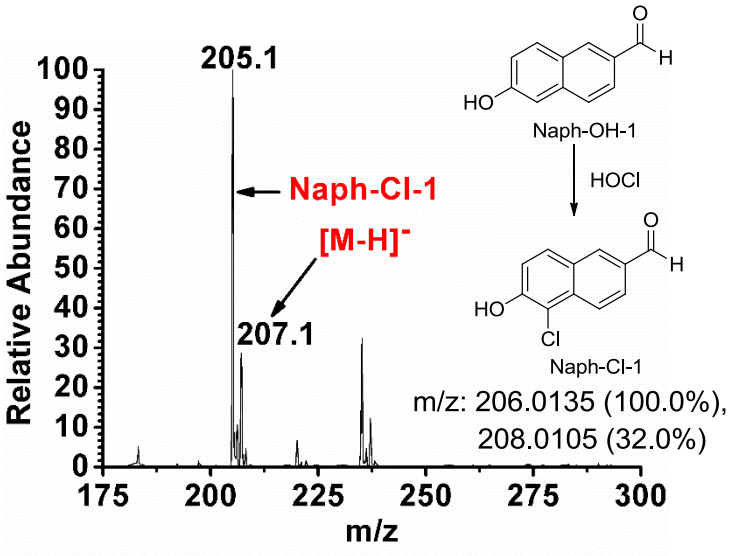



**Fig. S4** Mass spectrum of Naph-Cl-1 from the reaction of Naph-OH-1 and HOCl.

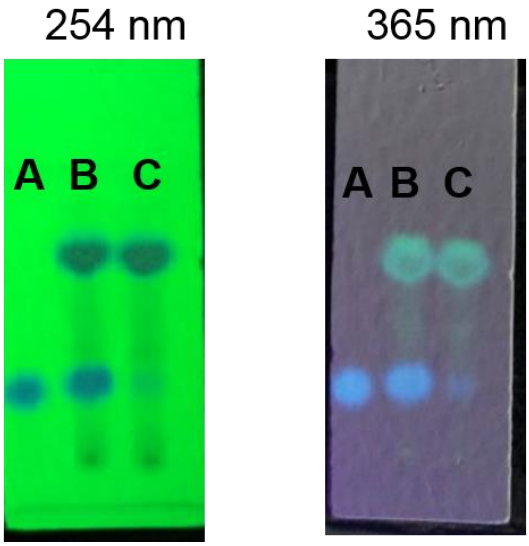



**Fig. S5** TLC images of the reaction between Naph-OH-1 and HOCl under a hand UV lamp. (A): Naph-OH-1; (B): mixture of (A) and (C); (C): reaction solution of Naph-OH-1 and HOCl.

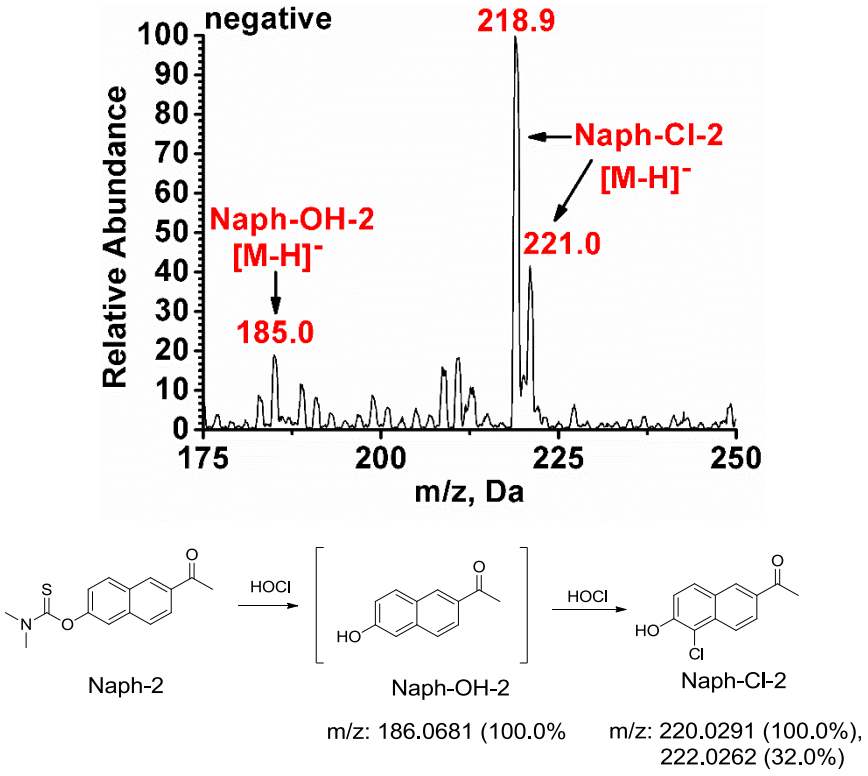



**Fig. S6** Mass spectrum of the reaction buffer of Naph-2 and HOCl in methanol.

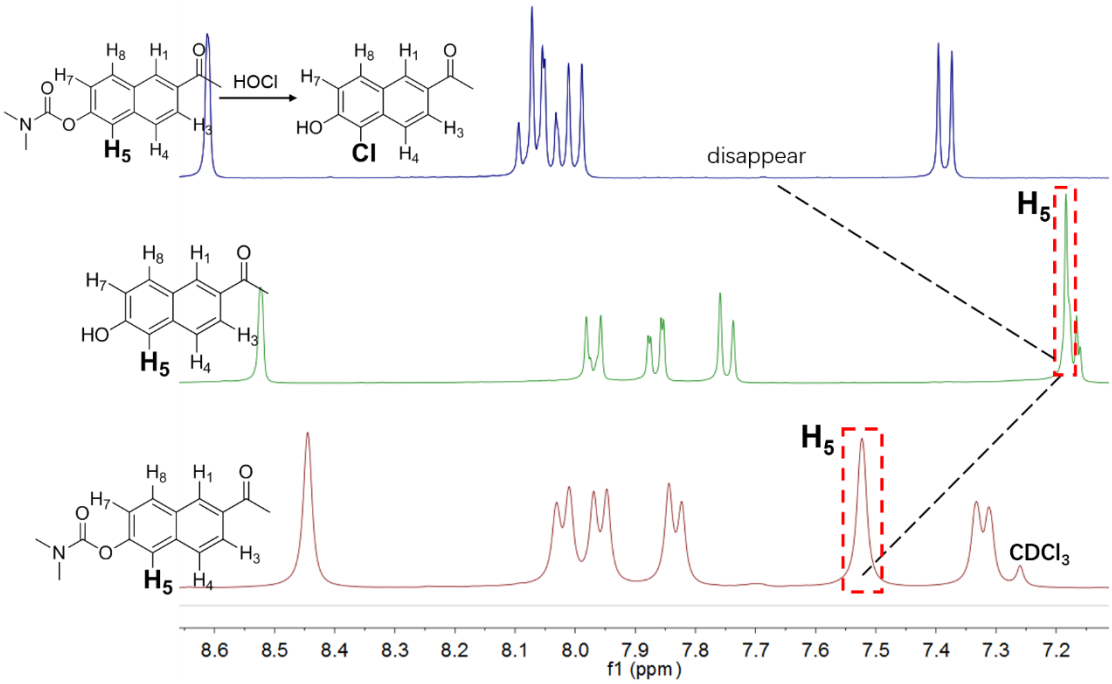



**Fig. S7** $^{1}$H NMR spectrum of Naph-2, Naph-OH-2 and the isolated Naph-Cl-2.

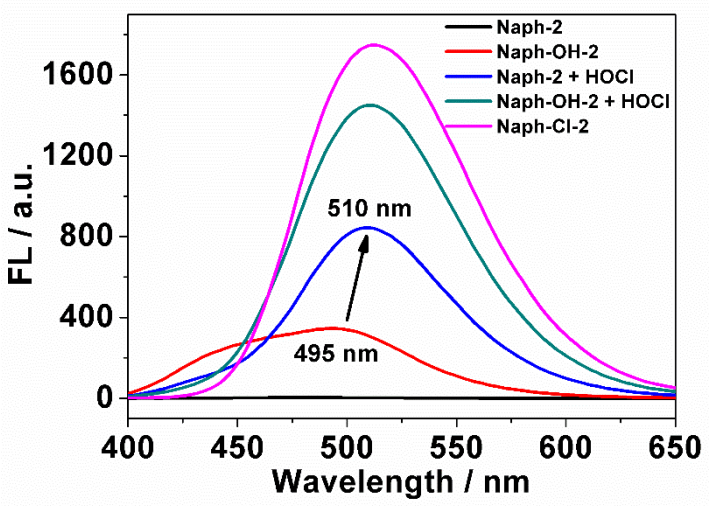



**Fig. S8** The differentiation of fluorescence spectrum of Naph-2, Naph-OH-2, reaction of Naph-2 and HOCl, reaction of Naph-OH-2 and HOCl, and Naph-Cl-2.

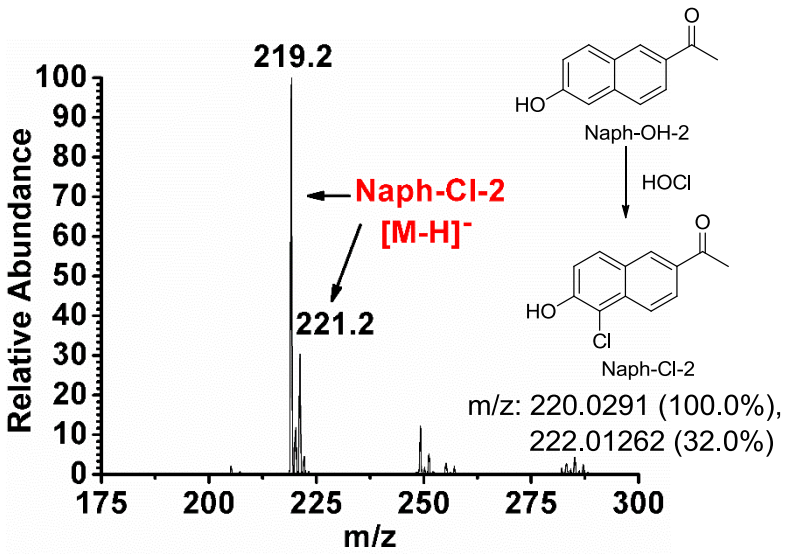



**Fig. S9** Mass spectrum of Naph-Cl-2 from the reaction of Naph-OH-2 and HOCl.

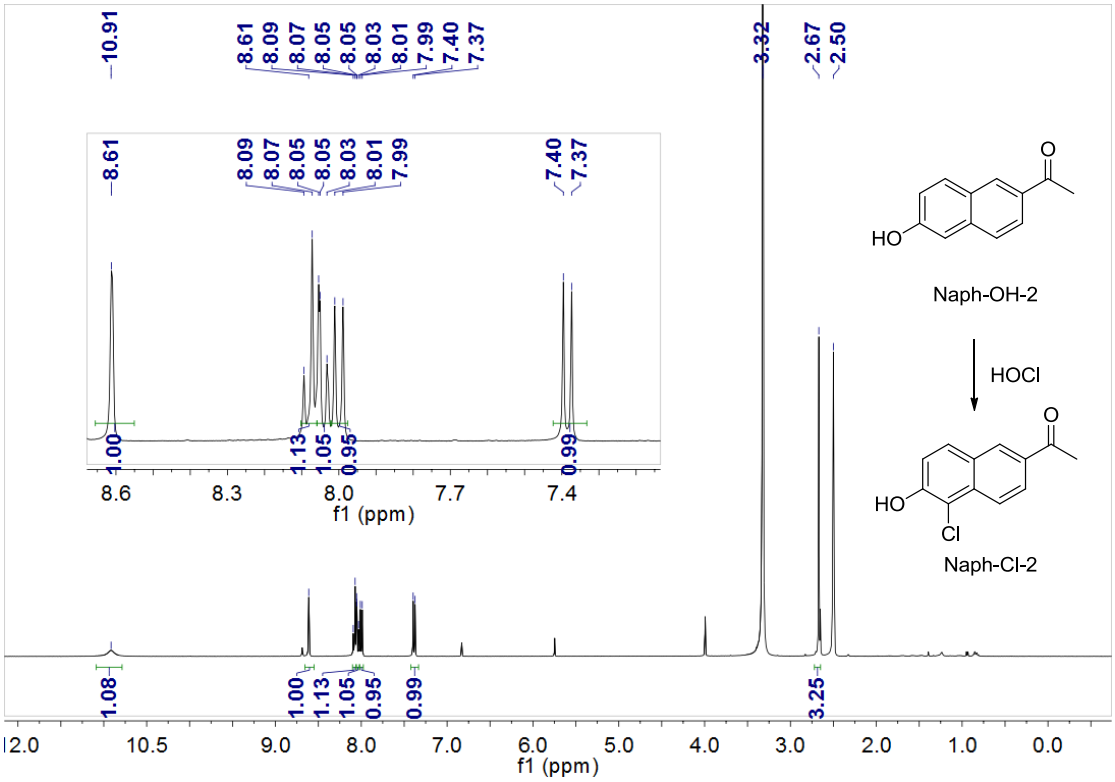



**Fig. S10** $^{1}$H NMR of Naph-Cl-2 from the reaction of Naph-OH-2 and HOCl ($d^{6}$-DMSO).

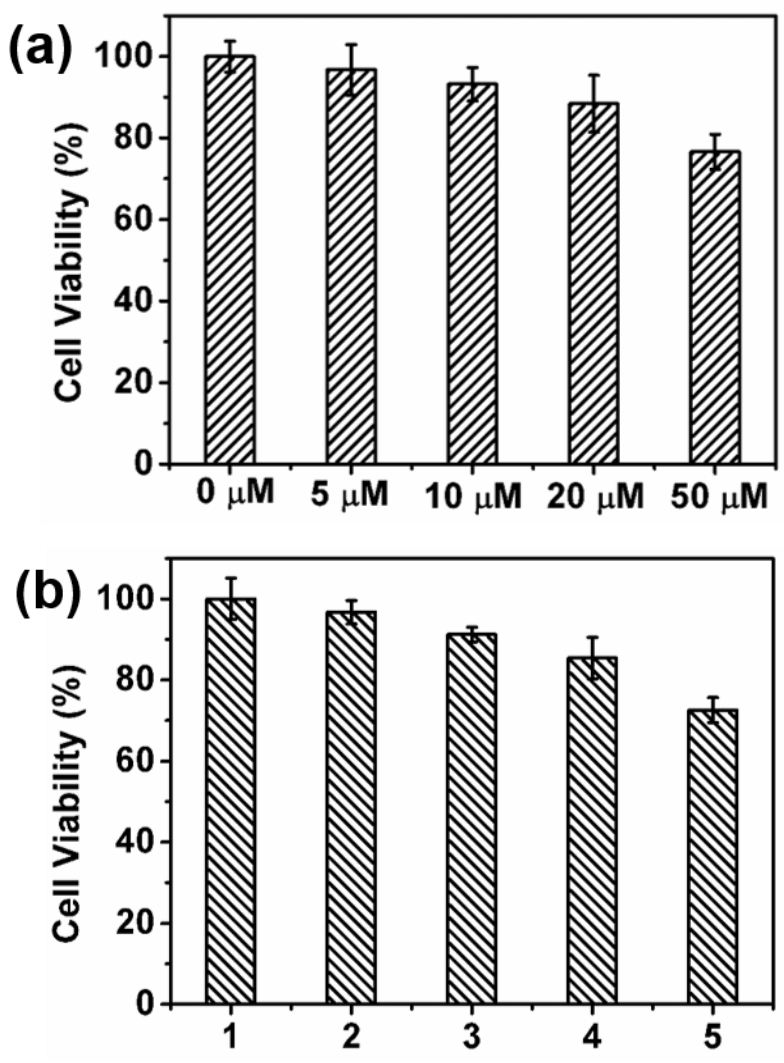



**Fig. S11** MTS assay with different concentrations of Naph-1 (a) and Naph-2 (b) in HeLa cells after 24 h incubation.

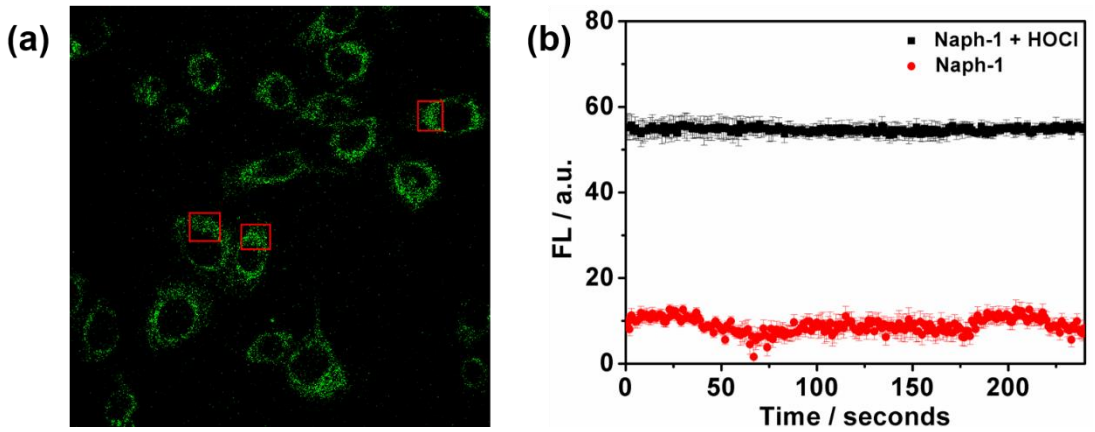



**Fig. S12** Stability test of Naph-1 under continuous irradiation at 405 nm with or without HOCl. (a) three regions of in the different cells; (b) the average fluorescence intensity and standard deviation in (a) with 1 s intervals for the duration of 4 min.

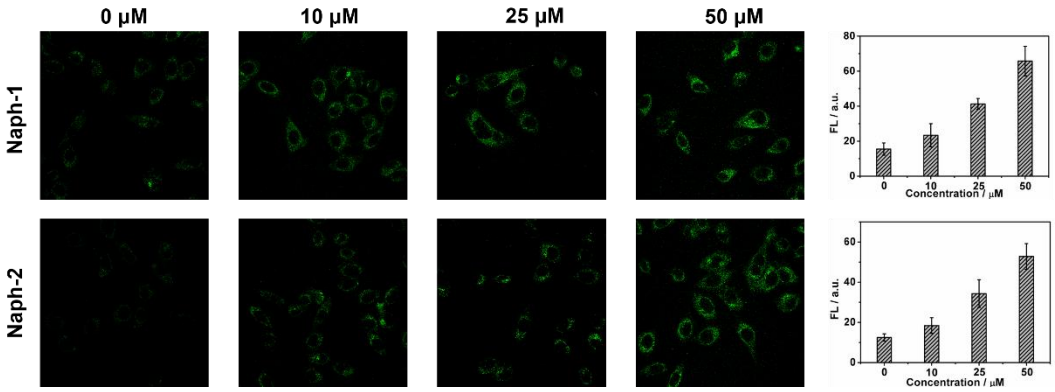



**Fig. S13** Confocal images of Naph-1 or Naph-2 (10 μM) in HeLa cell with pre-incubation of different concentrations of HOCl.

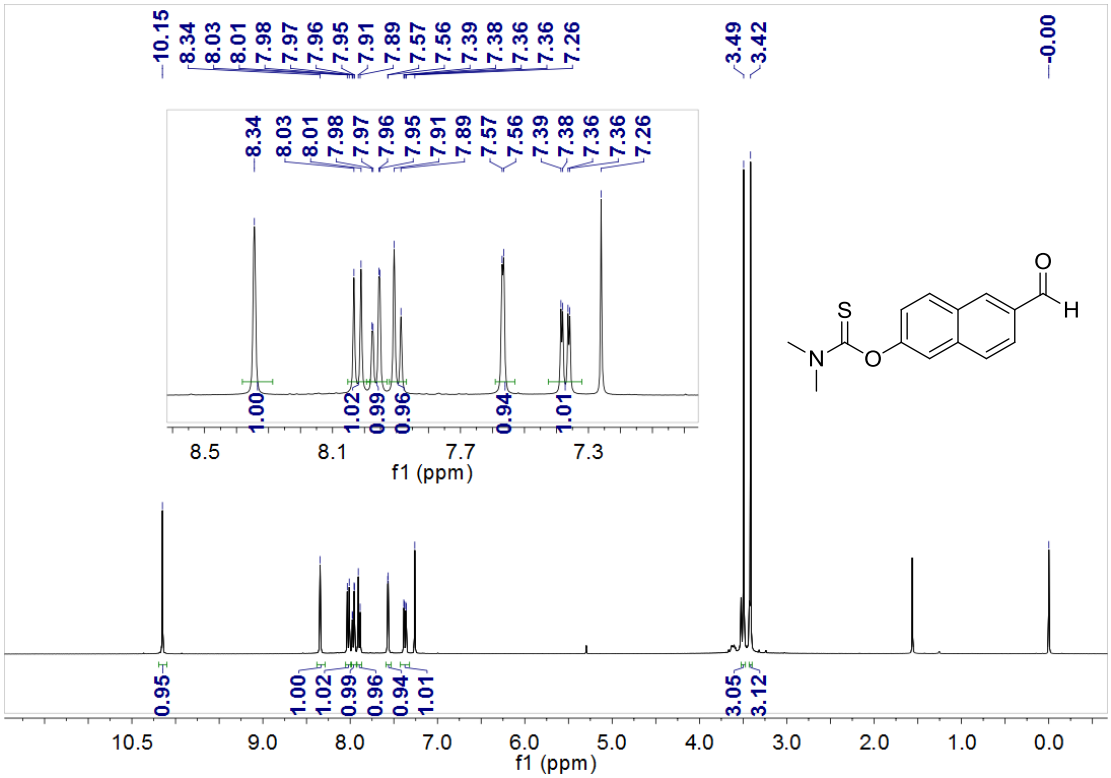



**Fig. S14** $^{1}H$ NMR spectrum of Naph-1 ($CDCl_3$).

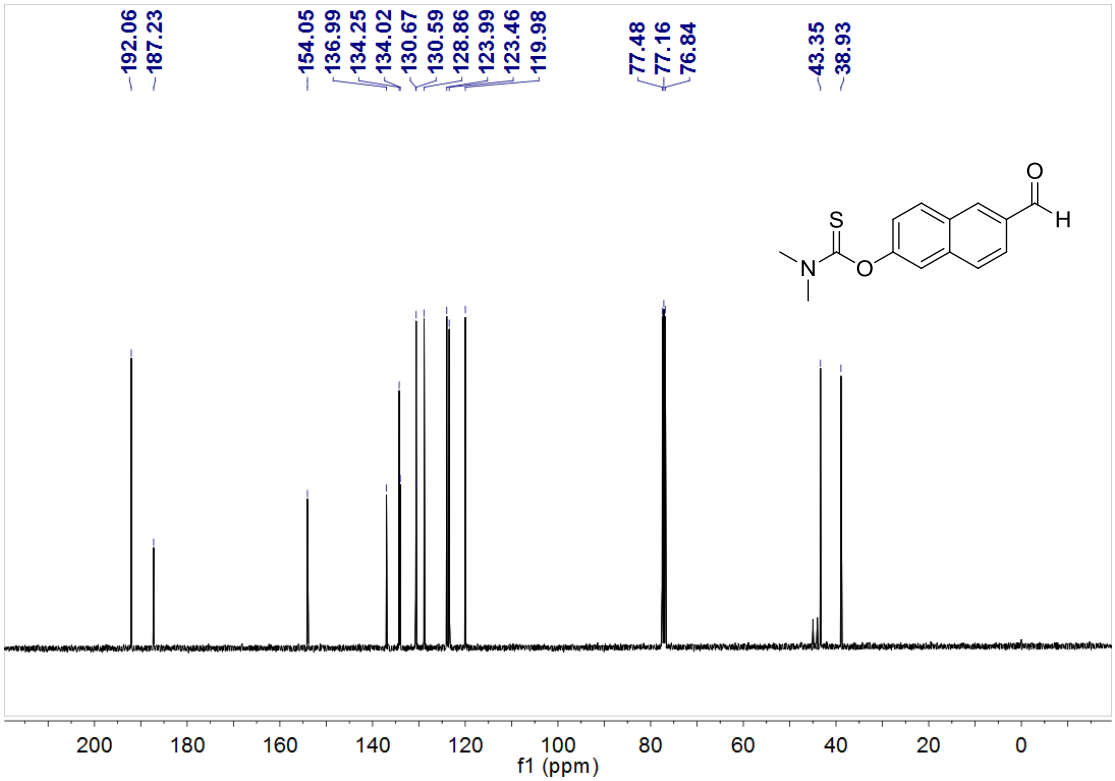



**Fig. S15** $^{13}C$ NMR spectrum of Naph-1 ($CDCl_3$).

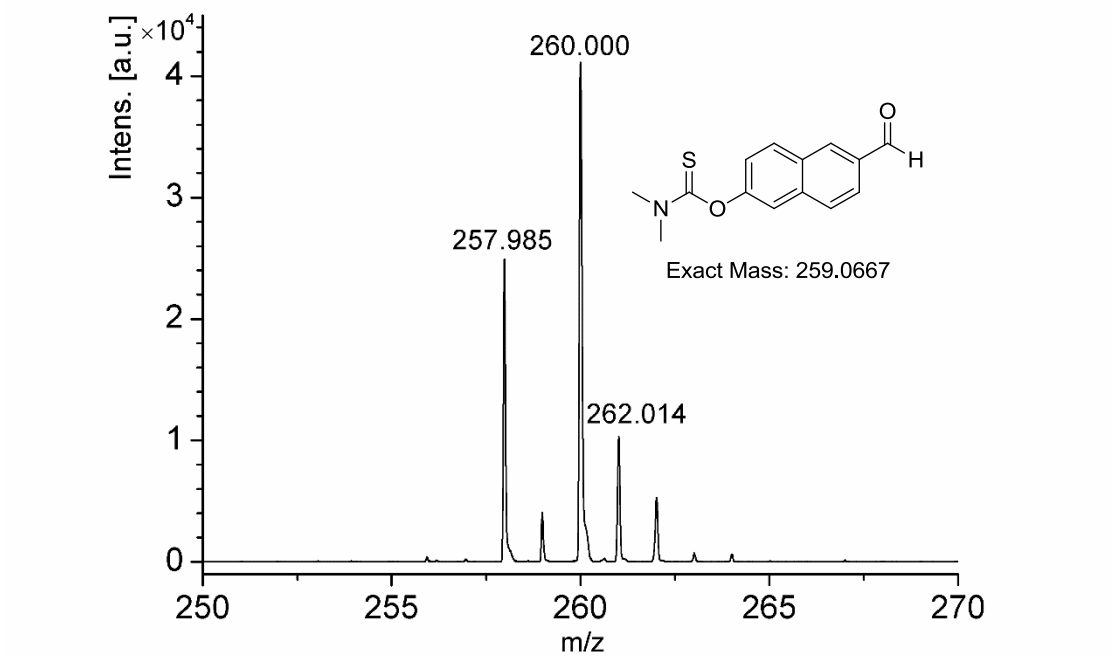



**Fig. S16** Mass spectrum of Naph-1.

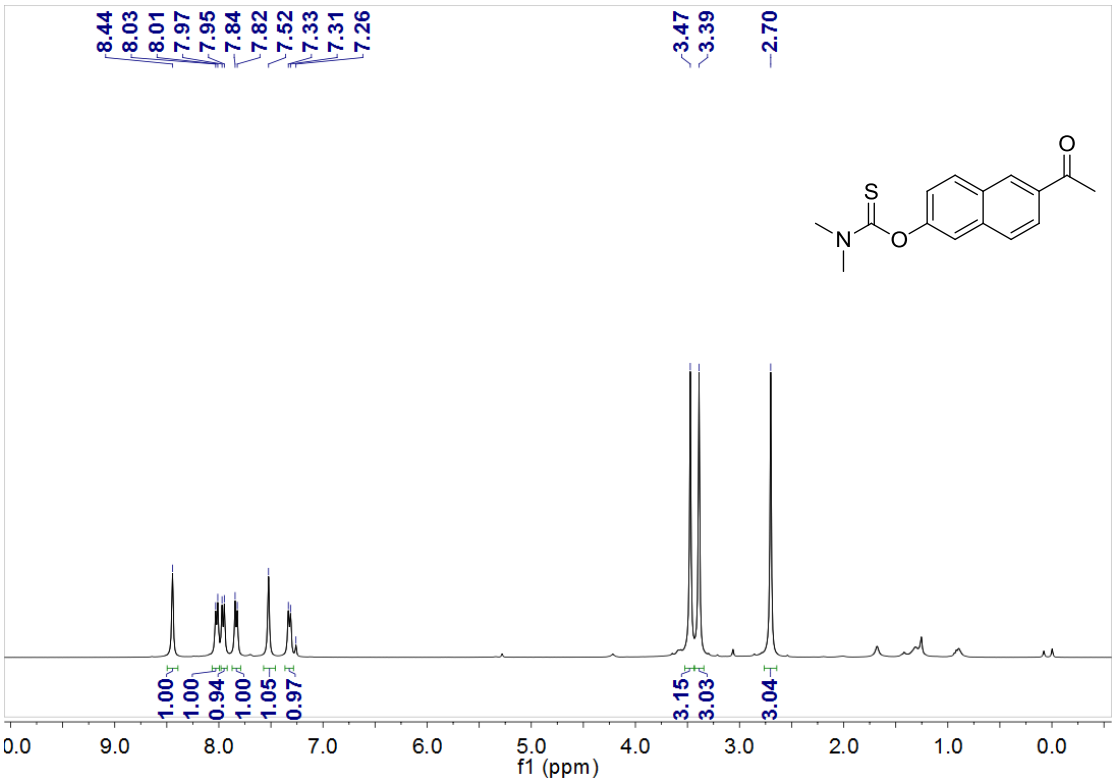



**Fig. S17** $^{1}$H NMR spectrum of Naph-2 ($CDCl_3$).

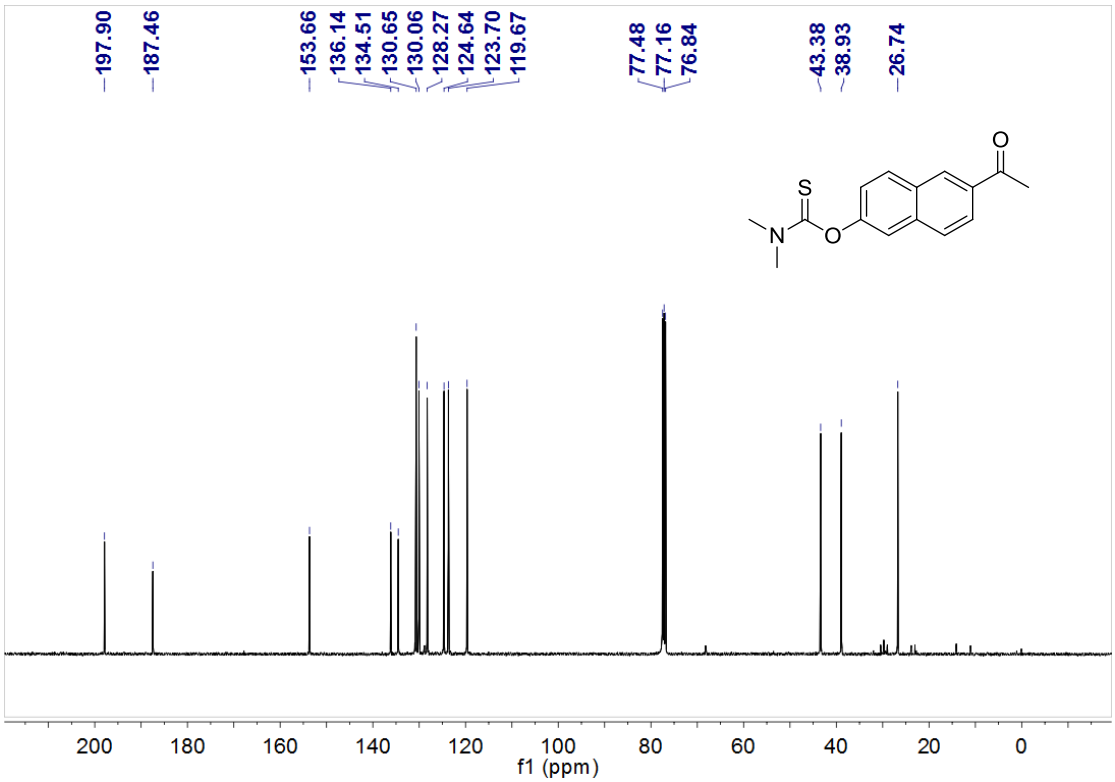



**Fig. S18** $^{13}$C NMR spectrum of Naph-2 ($CDCl_3$).

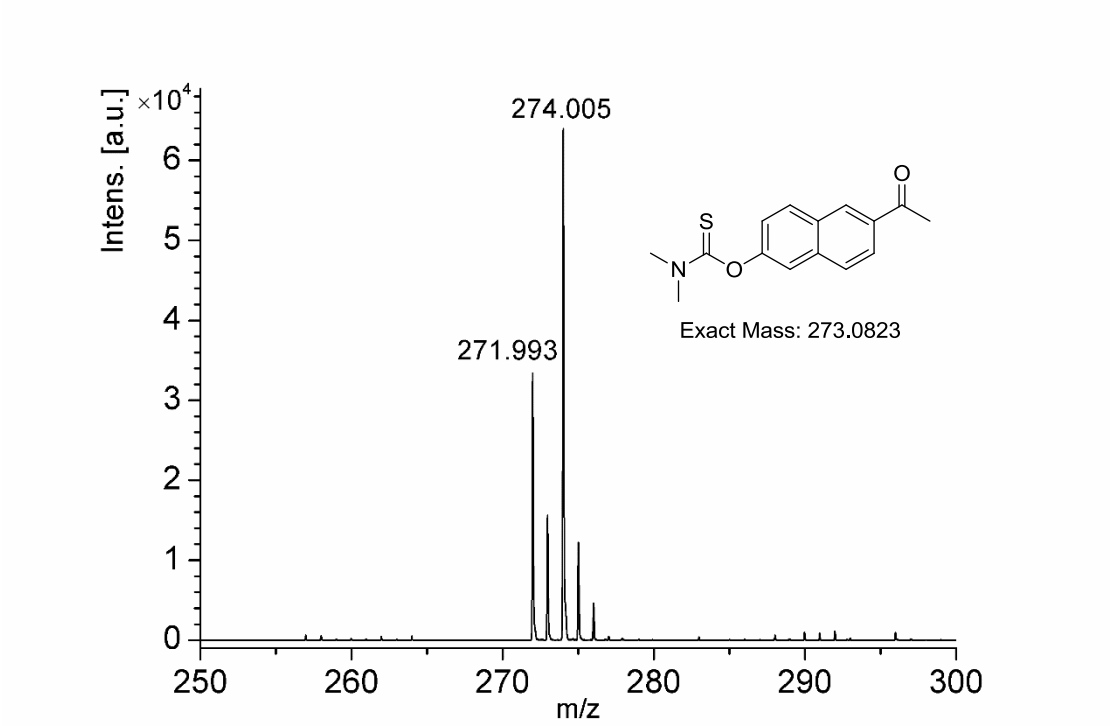



**Fig. S19** Mass spectrum of Naph-2.

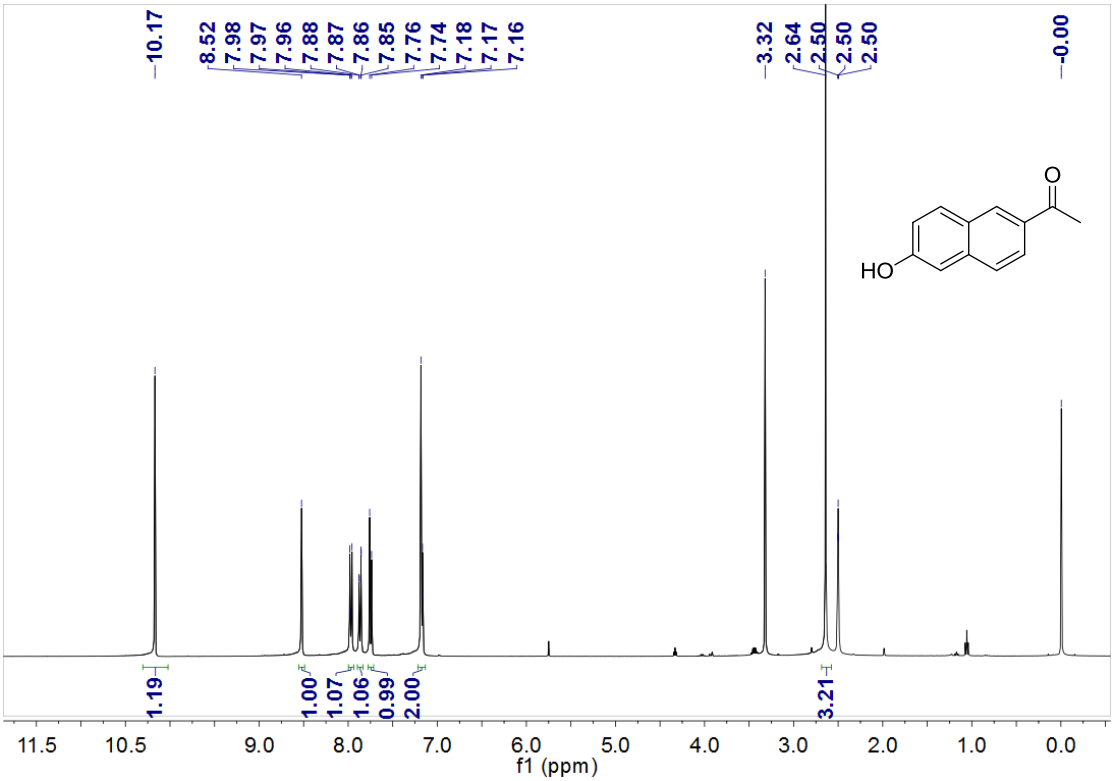



**Fig. S20** $^{1}$H NMR spectrum of Naph-OH-2 (*d*$^{6}$-DMSO).