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## Supporting information

### **A turn-on Schiff base fluorescent probe for exogenous and endogenous Fe<sup>3+</sup> ions sensing and bioimaging in living cells**

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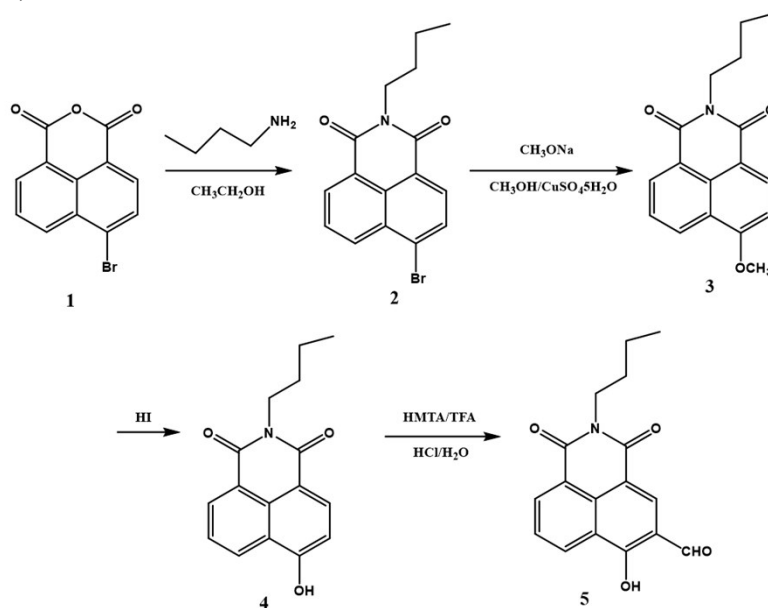
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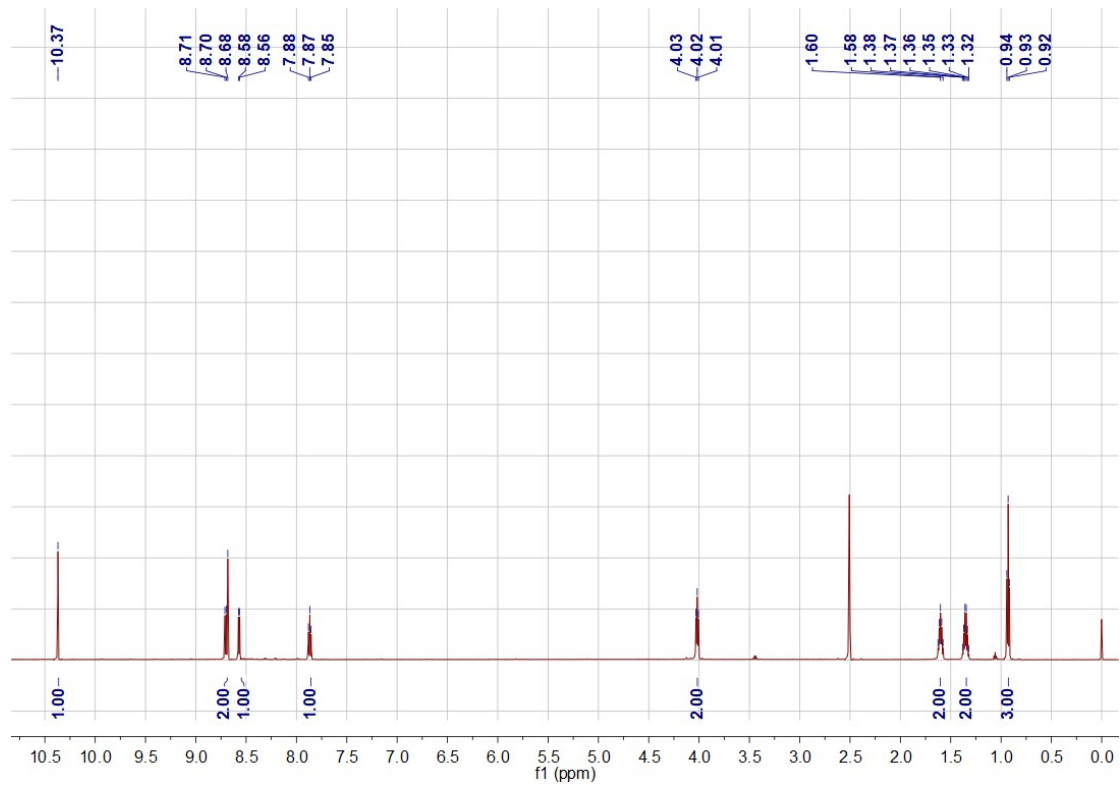
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## Synthesis of N-butyl-3-formyl-4-hydroxyl-1,8-naphthalene diformimide

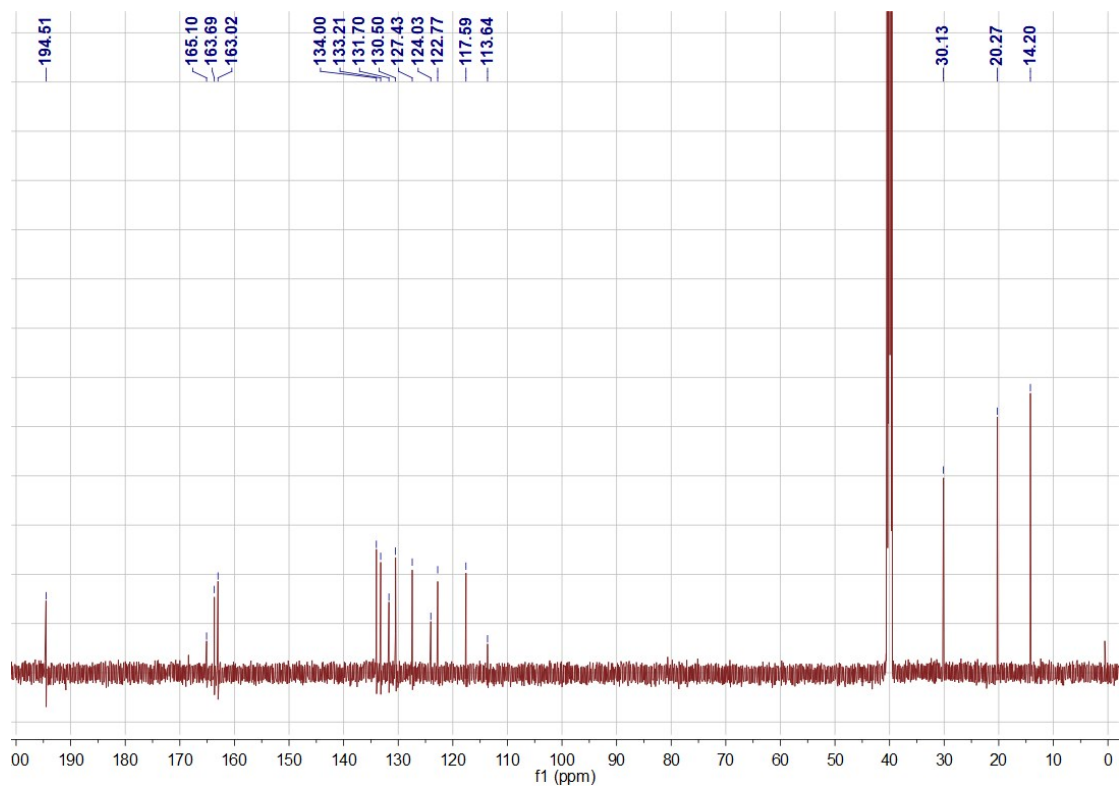
N-butyl-3-formyl-4-hydroxyl-1,8-naphthalene diformimide (**5**) was initially prepared using the literature method (Scheme S1). A mixture of 4-bromo-1,8-naphthalic anhydride (4.15 g, 15 mmol) and nbutylamine (1.10 g, 15 mmol) in ethanol was refluxed for 5 h. Then the mixture solution was cooled to room temperature and evaporated in vacuum and dried to obtain a solid product. Chromatography on a silica gel column in dichloromethane yields a pale-yellow solid product (**2**). Subsequently, compound **2** (3.31 g, 10 mmol) and sodium methoxide (0.54 g, 10 mmol) in methanol was refluxed for 8 h while  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  served as catalyst. Then the mixture was evaporated in vacuum and the residue was added HCl (1 mol/L, 15 mL) to crystallize out. The precipitated solid was filtered off and purified by chromatography on a silica gel column in dichloromethane to give a white product (**3**). What's more, compound **3** (2.83 g, 10 mmol) was added to 55% HI solution with refluxing at 140 °C for 7 h, and then the reaction was cooled to room temperature. Then the mixture was slowly poured into ice water. The precipitated solid was filtered off, washed with distilled water and dried under vacuum to give the product as pale yellow solid (**4**). Next, to a stirred trifluoroacetic acid (20 mL), compound **4** (2.69 g, 10 mmol) and hexamethylenetetramine (1.82 g, 13 mmol) was added. The solution was refluxed at 120 °C for 10h. When the remained solution was cooled to room temperature, it was slowly poured into the mixture solution of  $\text{CHCl}_3$  (50 mL) and HCl (1 mol/L, 50 mL) and then the mixture was warmed at room temperature whilst stirring overnight. After that, separate the organic phase and wash the aqueous phase three times with  $\text{CHCl}_3$  (20 mL  $\times$  3). The merged organic layer was dried with sodium sulfate and evaporated in vacuum to dry to a solid. Then the crude product was purified by chromatography on a silica gel column (ethyl acetate: petroleum ether = 1: 3), and give the desired product as a pale yellow solid **5** (1.39 g) in 47% yield.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ) (ppm): 10.37 (s, 1H), 8.70 (m, 2H), 8.56 (d, 1H), 7.87 (t, 1H), 4.02 (t, 2H), 1.60 (m, 2H), 1.35 (d, 2H), 0.93 (t, 3H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ) (ppm): 194.51, 165.10, 163.69, 163.02, 134.00, 133.21, 131.70, 130.50, 127.43, 124.03, 122.77, 117.59, 113.64, 30.13, 20.27, 14.20. HRMS (ESI):  $\text{C}_{17}\text{H}_{16}\text{NO}_4$  calcd. For 298.3175 (**5**+H), found 298.1055.



**Scheme S1** Synthesis of N-butyl-3-formyl-4-hydroxyl-1,8-naphthalene diformimide



**Fig. S1**  $^1\text{H}$  NMR spectra of N-butyl-3-formyl-4-hydroxyl-1,8-naphthalene diformimide.



**Fig. S2**  $^{13}\text{C}$  NMR spectra of N-butyl-3-formyl-4-hydroxyl-1,8-naphthalene diformimide.

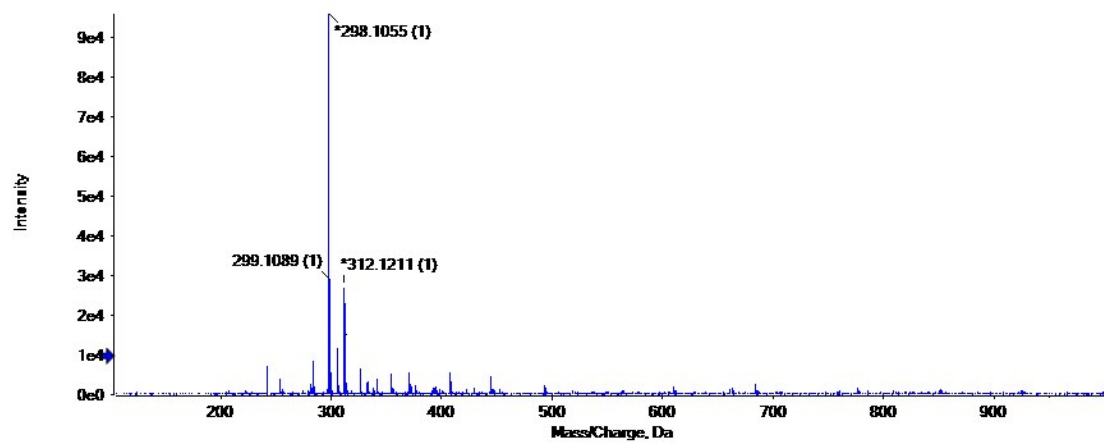
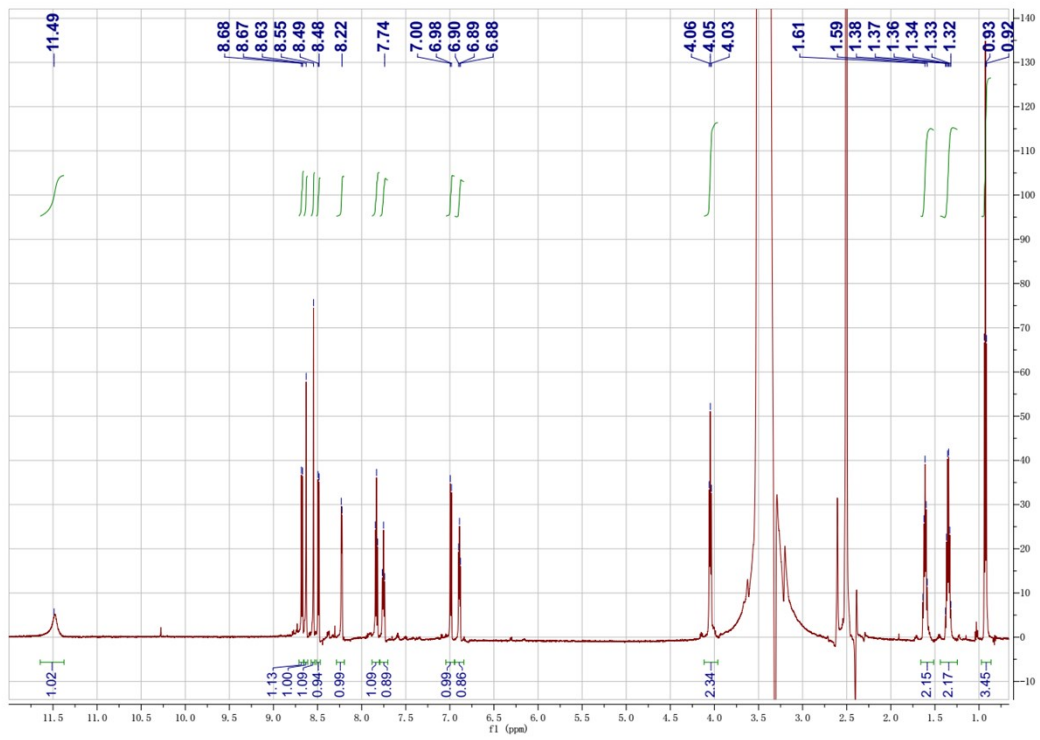


Fig. S3 ESI-MS spectra of N-butyl-3-formyl-4-hydroxyl-1,8-naphthalene diformimide.



**Fig. S4**  $^1\text{H}$  NMR spectra of NAH.

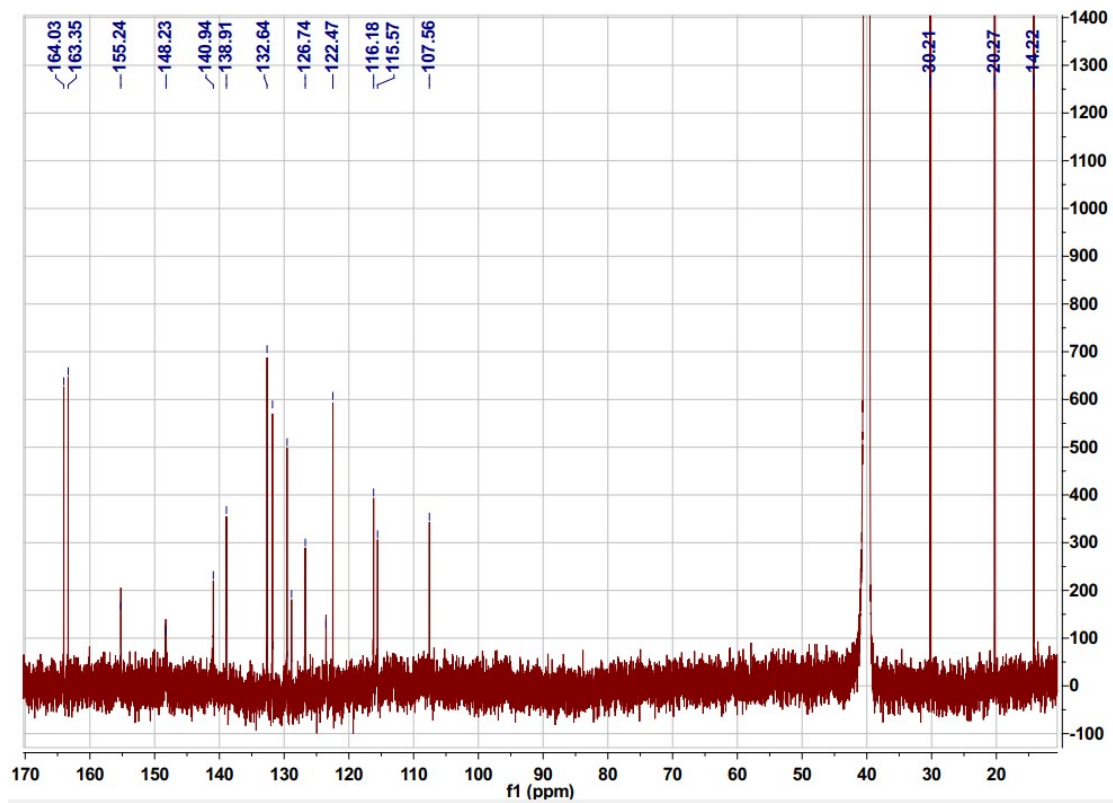
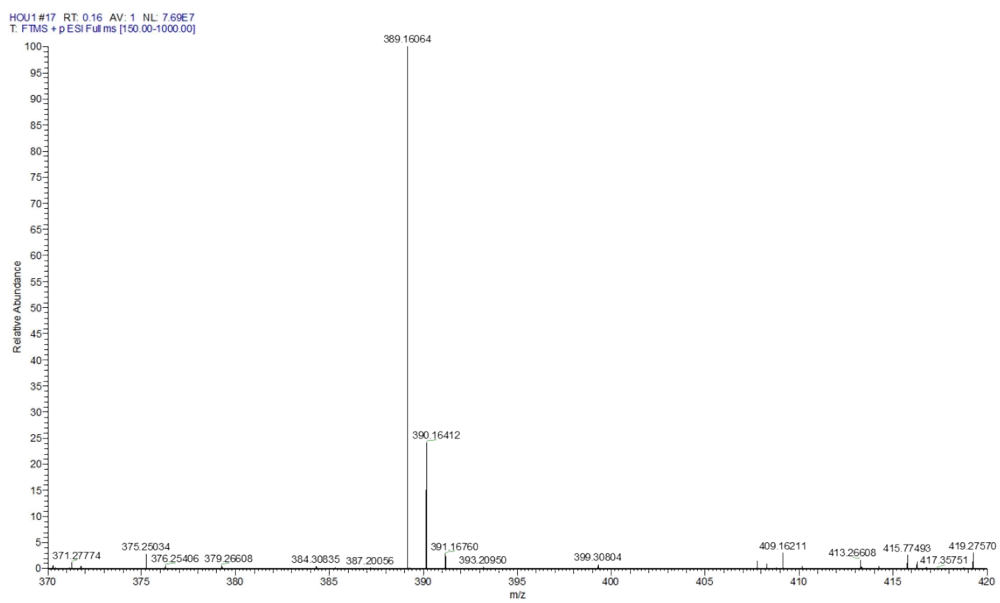


Fig. S5  $^{13}\text{C}$  NMR spectra of NAH.



**Fig. S6** ESI-MS spectra of **NAH**.

### Calculation of Detection Limit

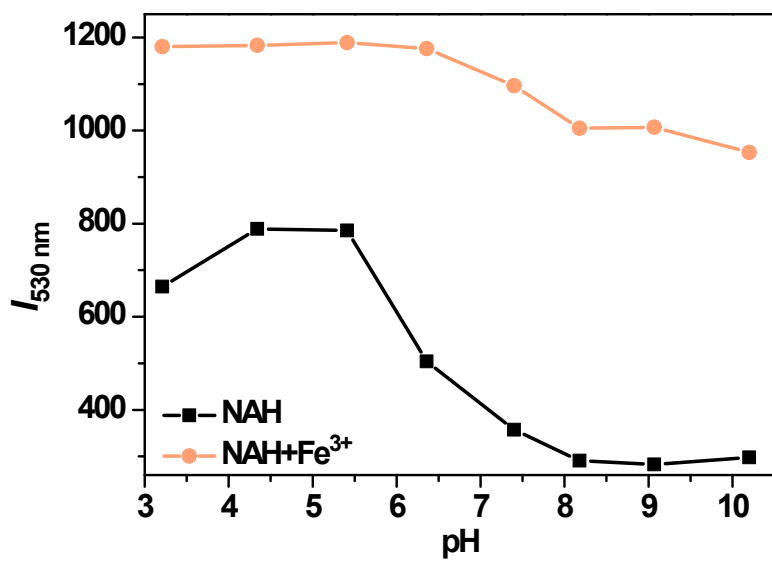
The detection limit can be calculated via the  $3\sigma$  method following the formula S1.

$$D = 3 s / b \quad (S1)$$

$s$  is the standard deviation of 10 blank samples, and the  $b$  is the slope of the calibration curve for the fluorescence intensity against concentration of analyte.

In this experiment, the  $s$  has been calculated as 2.56 %, the  $b$  is  $200.67 \times 10^6$  L/mol (see the Fig. 3b). Following the formula above, the  $D$  could be calculated to  $3.8 \times 10^{-8}$  mol/L.





**Fig. S7** pH effect on fluorescence intensity of NAH (5  $\mu$ M) at 530 nm ( $I_{530 \text{ nm}}$ ) in the absence and presence of Fe<sup>3+</sup>.

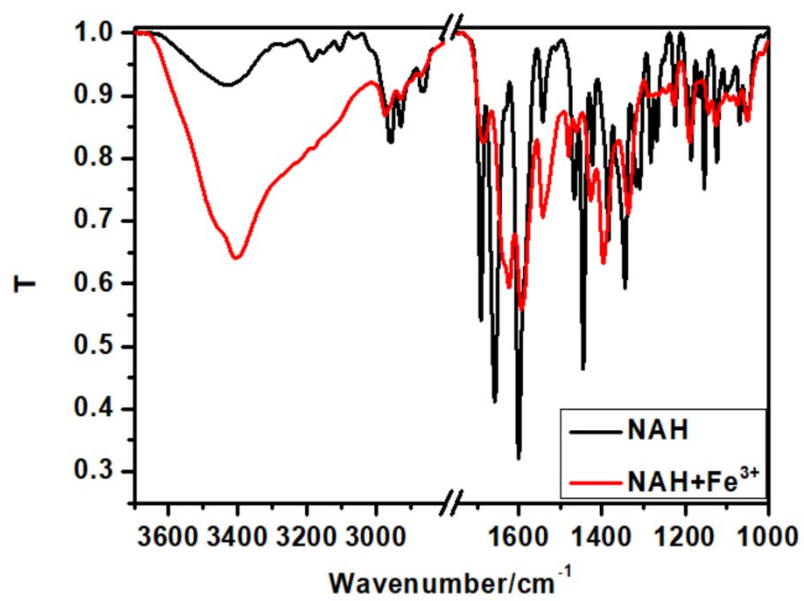
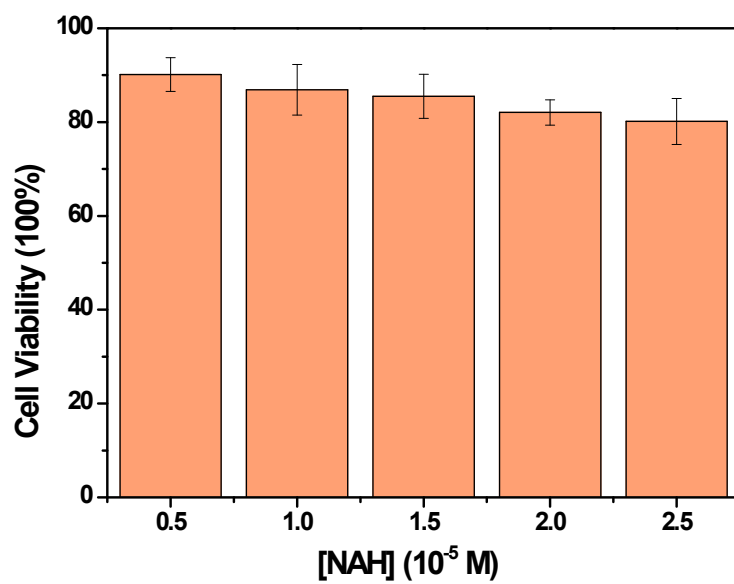
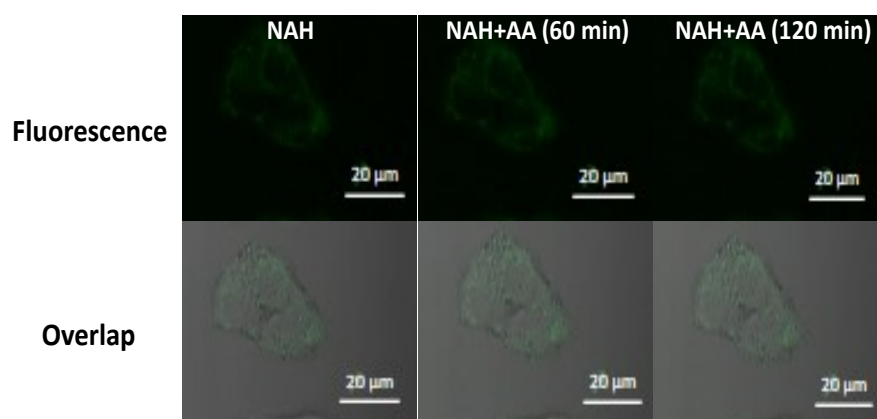


Fig. S8 FTIR spectra of NAH and Fe<sup>3+</sup>-NAH complex.



**Fig. S9** Cell viability test of ble-7420 cells treated with various concentrations of the NAH (from 0 to 25  $\mu$ M) for 24 h.



**Fig. S10** Real time fluorescence images of bel-7420 cells treated with **NAH** (10  $\mu$ M) for 20 min, and followed by **AA** (1 mM) treatment and imaged after: 0 min, 60 min, 120 min.