Fluorescent probe based on rosamine with pyridinium unit for hydrogen sulfide detection in mitochondria

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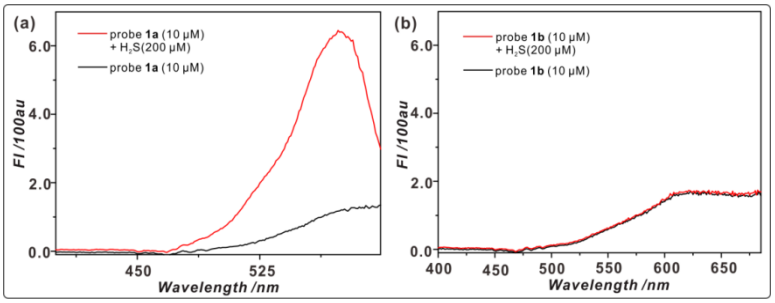

Fig. S1 Excitation spectra of probes **1a**−**b** (10 μM, λ_{em} = 604 nm) towards H_2S in PBS buffer (20 mM, pH=7.4) containing 10% DMSO.

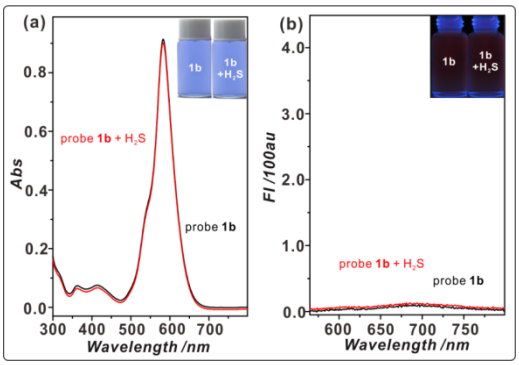

Fig. S2 Absorption and emission titration of probe **1b** (10 μM) towards H_2S (20 equivalent) in PBS buffer (20 mM, pH=7.4) containing 10 % DMSO. (a) Absorption spectra; (b) emission spectra.

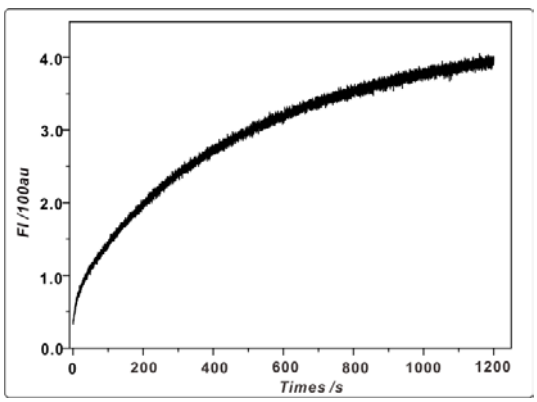

Fig. S3 Time-dependent fluorescence-intensity changes of probe **1a** (10 μM) towards the H_2S (20 equivalent).

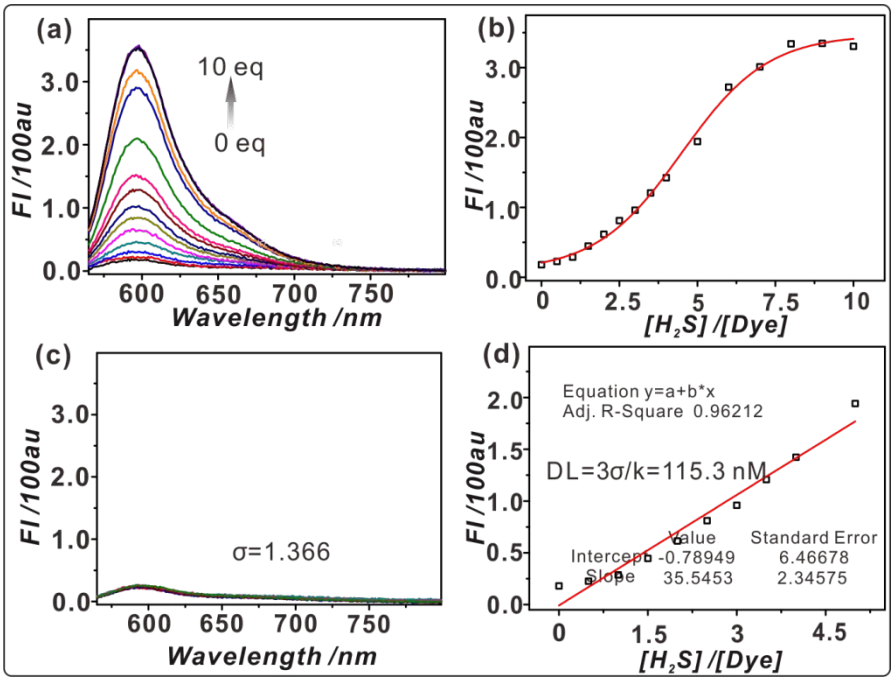

Fig. S4 Determinations of detection limit of probe **1a**. (a) Fluorescence spectra of probe **1a** (1 μM) in PBS buffer solution in presence of different concentrations of H_2S (0−10 equivalent); (b) fluorescence intensity changes at 604 nm of probe **1a** with the amount of H_2S (0−10 equivalent); (c) Fluorescence spectra of probe **1a** (1 μM) in PBS buffer solution in absence of H_2S and standard deviation of blank measurements; (d) plot of the fluorescence intensity of **1a** upon addition of H_2S (0−5 equivalent).

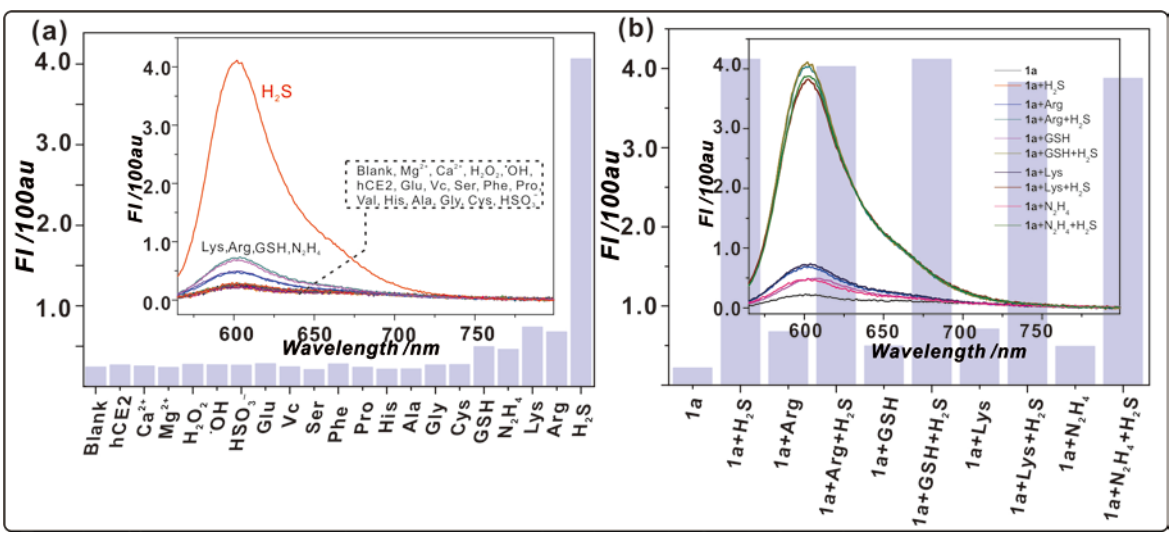

Fig. S5 Selective and competition experiments of probe **1a**. (a) probe **1a** (10 μM) towards the relevant species (hCE2:5μg/mL, GSH:3mM, others: 500 μM); (b) probe **1a** (10 μM) in the presence of only 500 μM H_2S, 500 μM H_2S + 500 μM Arg, 500 μM H_2S + 3mM GSH, 500 μM H_2S + 500 μM Lys, , 500 μM H_2S + 500 μM N_2H_4.

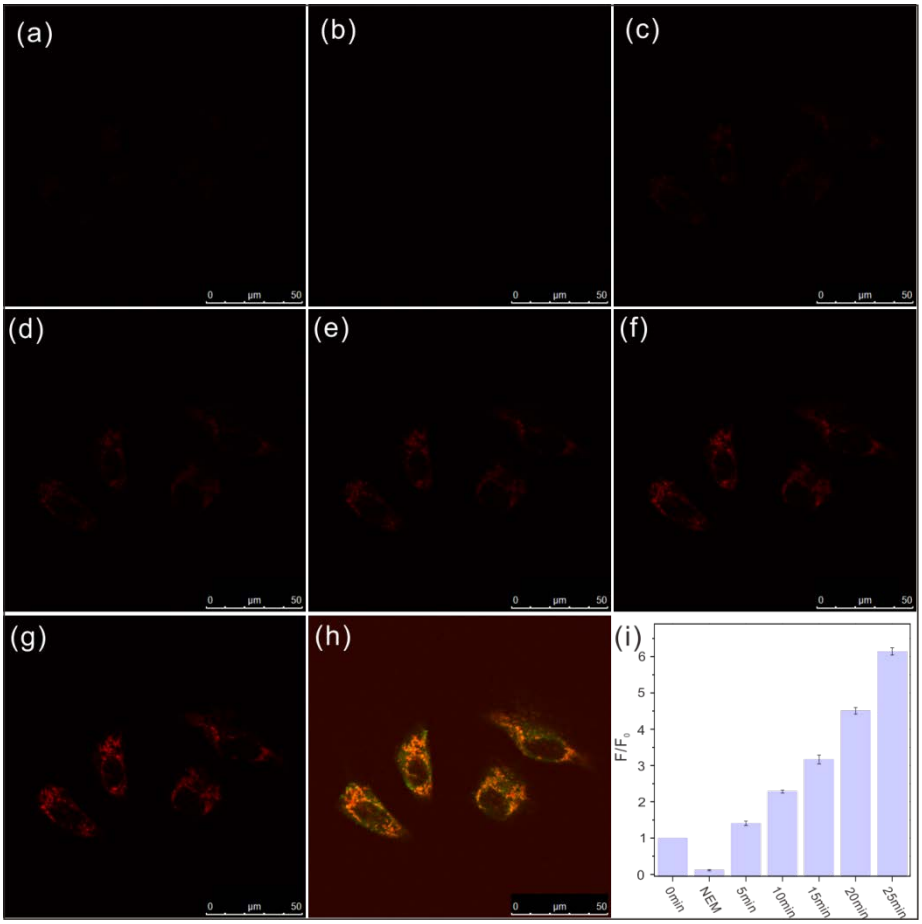

Fig. S6 Fluorescence confocal images of HeLa cancer cells with probe **1a** (200 nM) and standard MitoTracker Green FM (50 nM). (a) Confocal images (red channel) of cells with probe **1a** before addition of H_2S; (b) Confocal images of cells with 500 μM NEM for 30 min after addition probe **1a (**200 nM); (c−g) Confocal images of cells with probe **1a** after addition of H_2S (4 μM) for 5 min, 10 min, 15 min, 20 min, 25 min; (h) Merged images with enhanced background light of (a) and MitoTracker Green FM (50 nM); (i) The fluorescence intensity ratio of cell imaging with probe **1a (**200 nM) after addition of H_2S (4 μM) for different times.

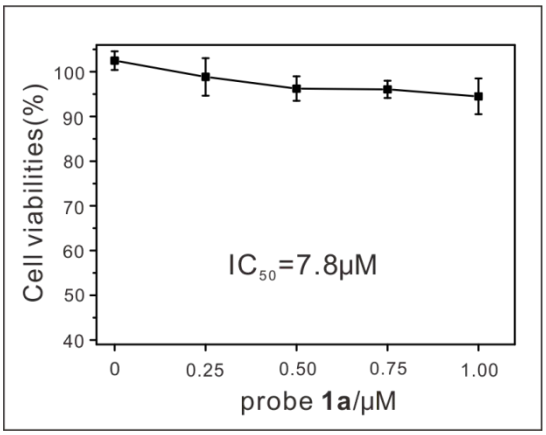

Fig. S7 Percentages of cell viabilities of HeLa cells after treatment with probe **1a** for 6 hours. And IC_{50} was calculated to 7.8μM. Cell viabilities were assayed by the CCK-8 method.

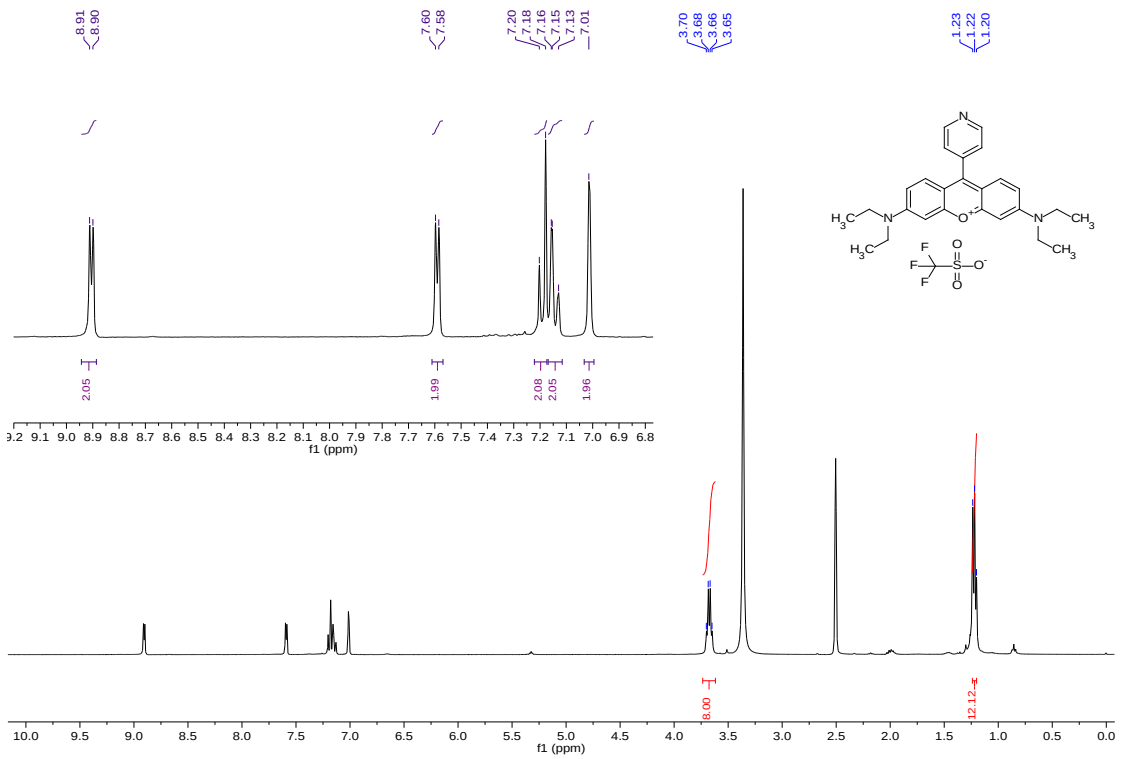

Fig. S8 ^{1}H NMR spectrum of **4**.

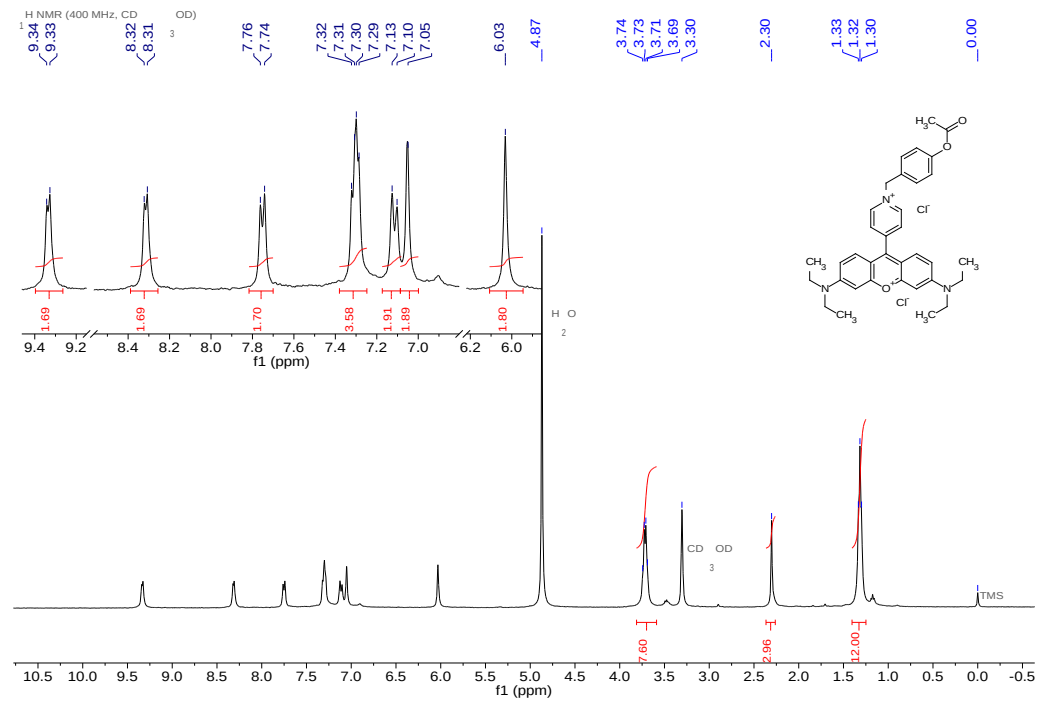

Fig. S9 ^{1}H NMR spectrum of **1a**.

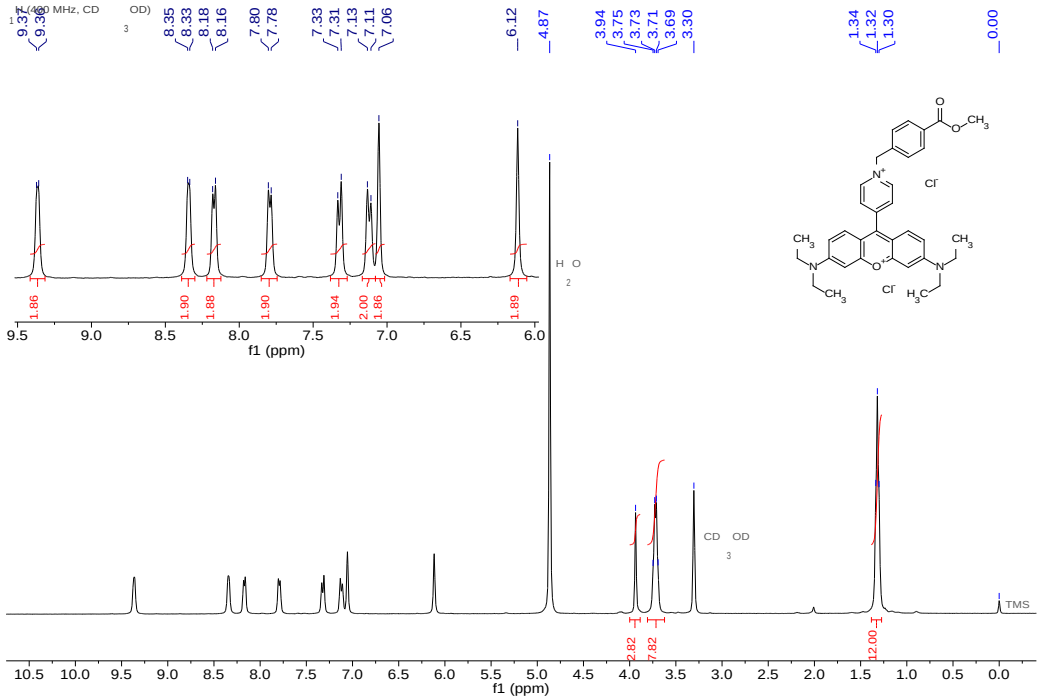

Fig. S10 ^{1}H NMR spectrum of **1b.**.

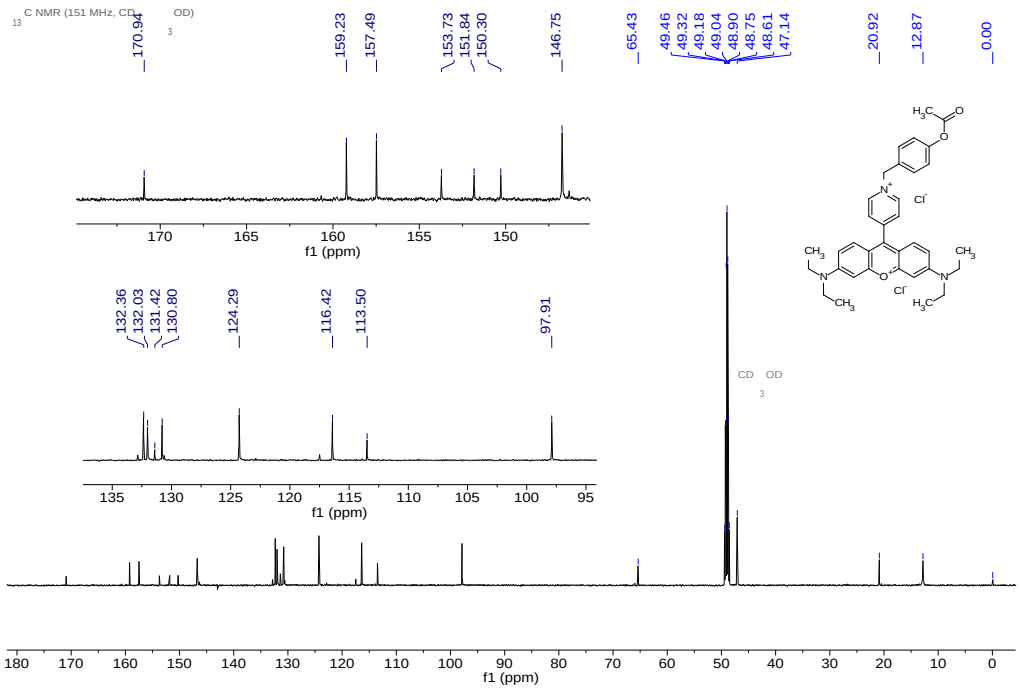

Fig. S11 ^{13}C NMR spectrum of **1a.**

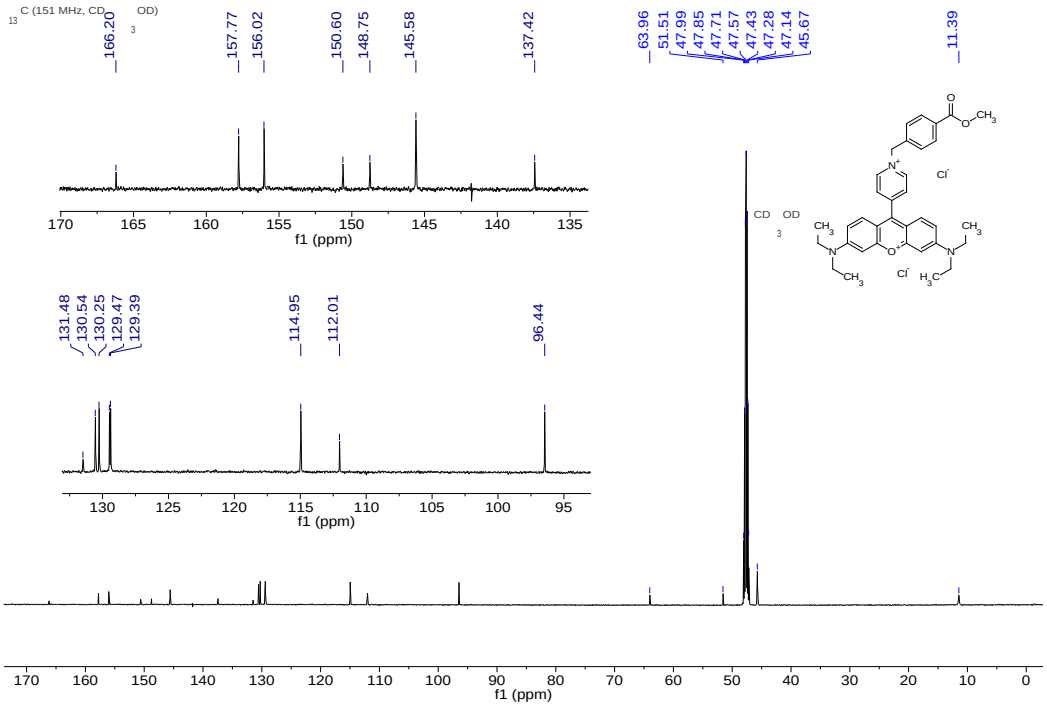

Fig. S12 13C NMR spectrum of **1b.**

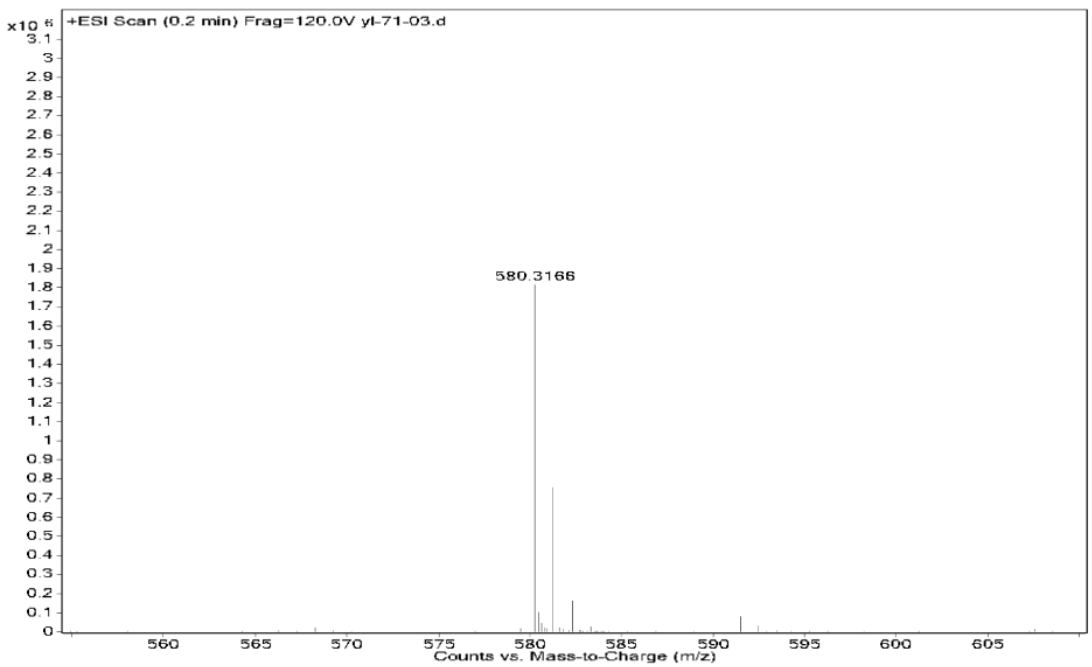

Fig. S13 HRMS spectrum of probe **1a** (ESI^+).

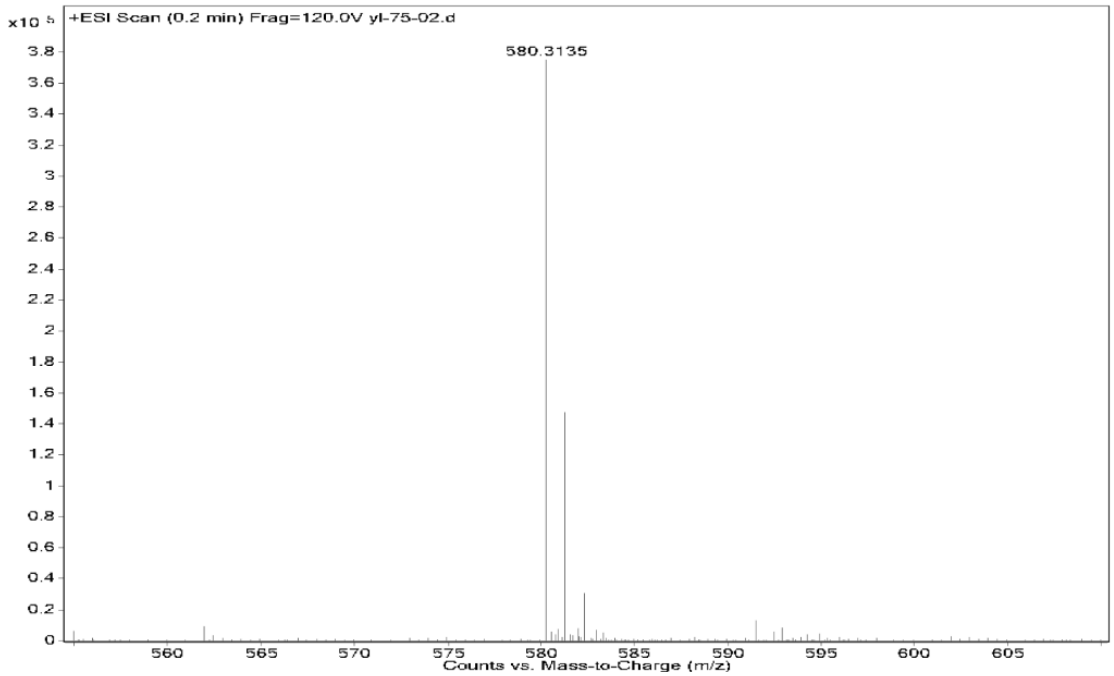

Fig. S14 HRMS spectrum of probe **1b** (ESI^+).