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Supporting information

A Fluorescent Probe for Intracellular Cysteine Overcoming the Interference by Glutathione

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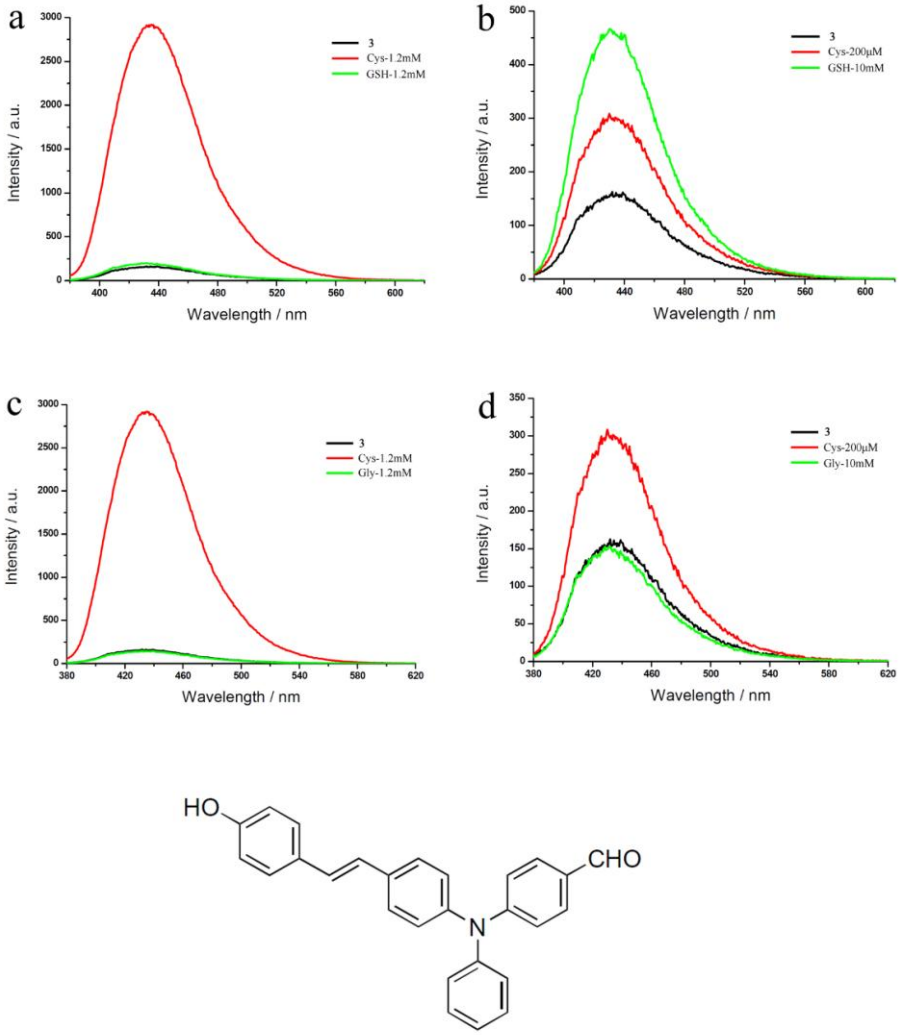
Results and discussion



Structure of molecule **3**

Fig. S1 Fluorescent spectra of probe **3** (10 μM) and in the presence of various analytes in EtOH/PBS buffer solution (v/v, 4:1), λ_{ex} = 365 nm. (a) 1.2 mM Cys and 1.2 mM GSH; (b) 200 μM Cys and 10 mM GSH; (c) 1.2mM Cys and 1.2mM Gly; (d) 200 μM Cys and 10 mM Gly.

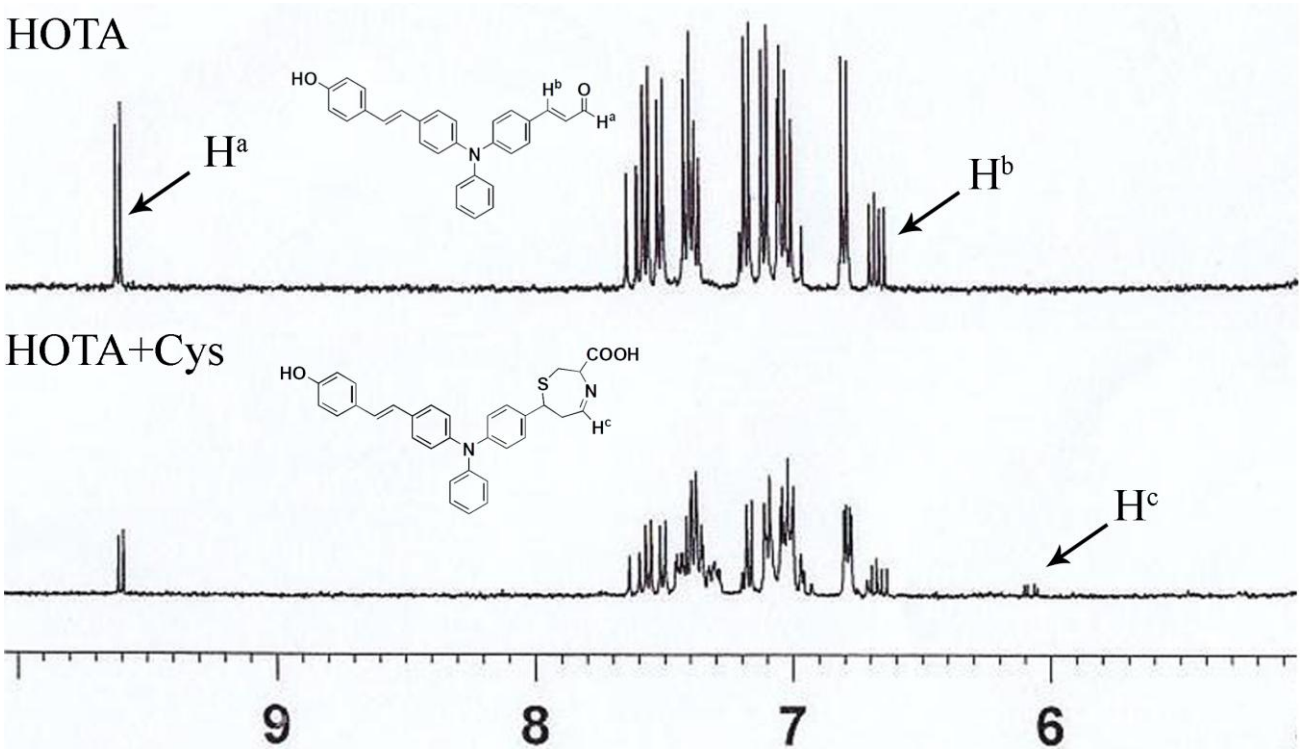


Fig. S2 ^{1}H NMR spectra of **HOTA** itself and in the presence of Cys in CD_3OD.

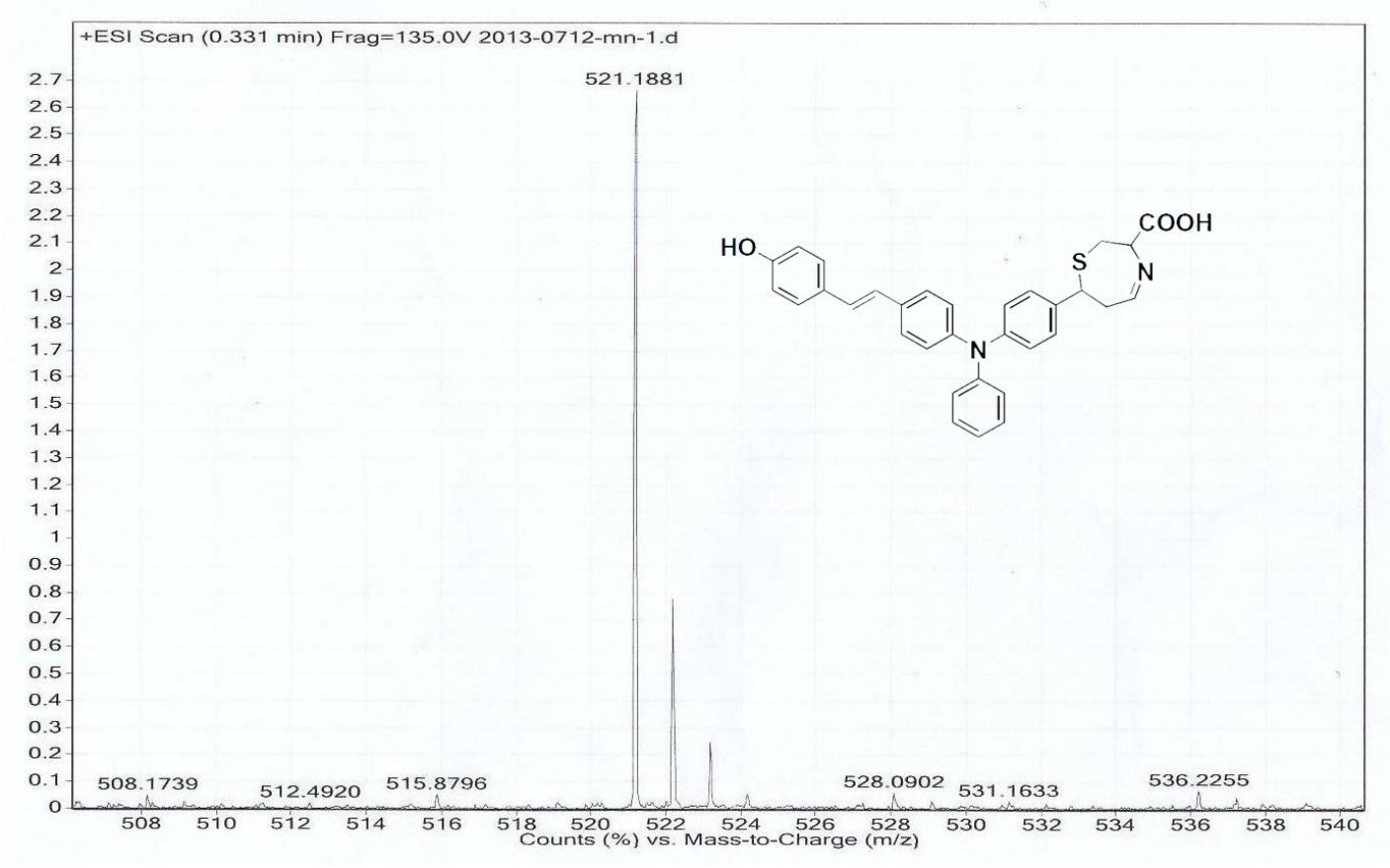


Fig. S3 HRMS spectra of mixture of **HOTA** and Cys in the mixed solvents of EtOH and H_2O (v/v, 1:1).

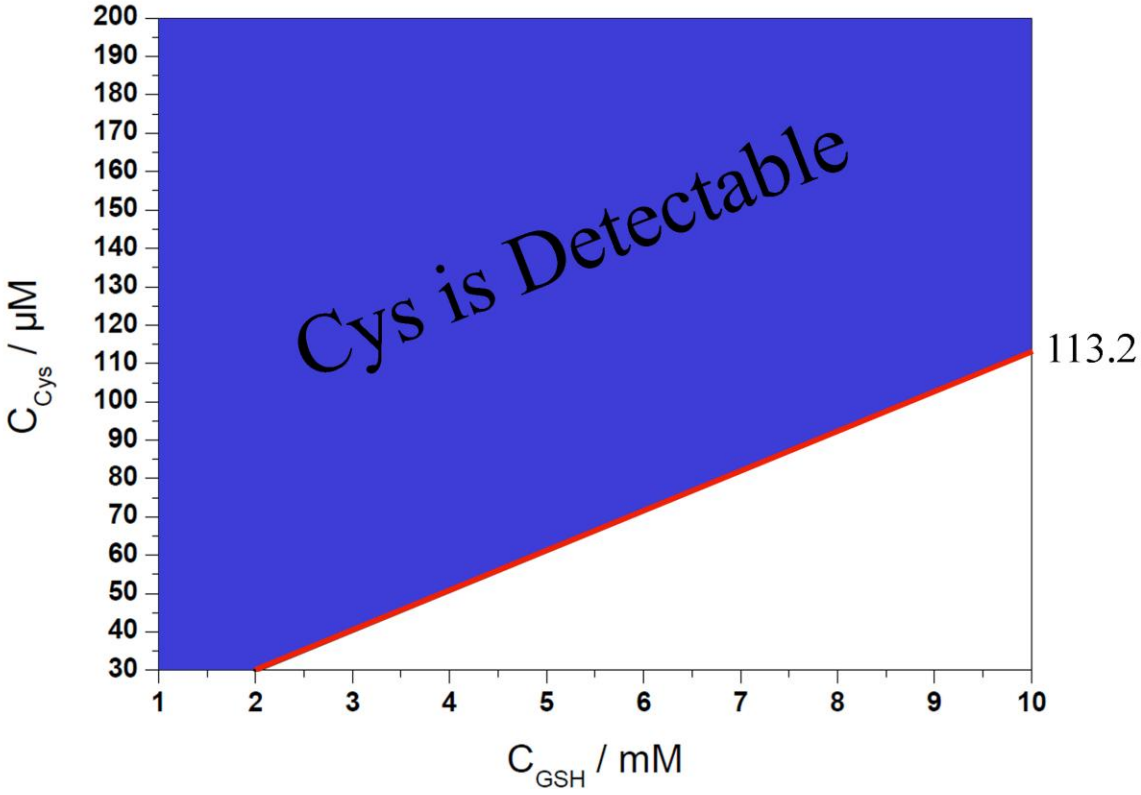


Fig. S4 Intracellular concentration ranges of GSH (1~10 mM) and Cys (30~200 μM). The fluorescence intensity of **HOTA**+Cys is 3 times stronger than that of GSH when their concentrations are in the area marked with blue colour (C_{HOTA} = 10 μM).

Note: According to our titration experiments, the relationship between fluorescence intensity at 450 nm and concentration of Cys/GSH could be depicted by two equations:

$I_{Cys} \geq 86.57 + 7.937 * C_{Cys}$ (μM); $I_{GSH} = 53.18 + 27.5 * C_{GSH}$ (mM).

where I_{Cys} and I_{GSH} are the fluorescence intensities of HOTA in the presence of Cys and GSH, respectively, and $30 \leq C_{Cys} \leq 200$, $1 \leq C_{GSH} \leq 10$. Therefore, the interference from GSH could be ignored when $I_{Cys} > 3I_{GSH}$ or $86.57 + 7.937 * C_{Cys}$ (μM) $> 3 * [53.18 + 27.5 * C_{GSH}$ (mM)]. Finally, a new equation can be deduced: $7.937 * C_{Cys}$ (μM) $- 82.5 * C_{GSH}$ (mM) $- 72.97 > 0$, and $30 \leq C_{Cys} \leq 200$, $1 \leq C_{GSH} \leq 10$. This new equation can be transferred to a two-dimension picture that presented above.

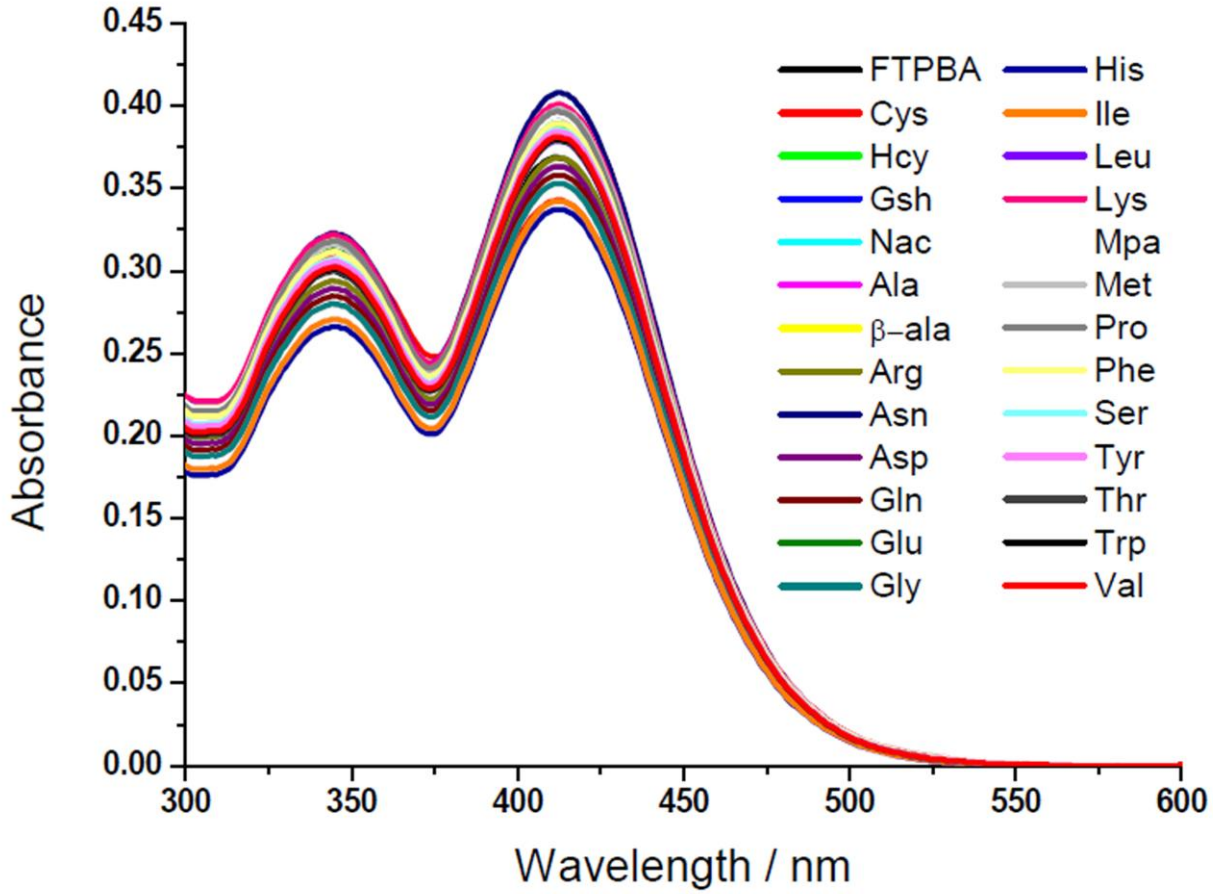


Fig. S5 Absorbance spectra of **HOTA** (10 μM) itself and in the presence of 200 μM of various analytes EtOH/PBS buffer solution (v/v, 1:1, pH 7.4).

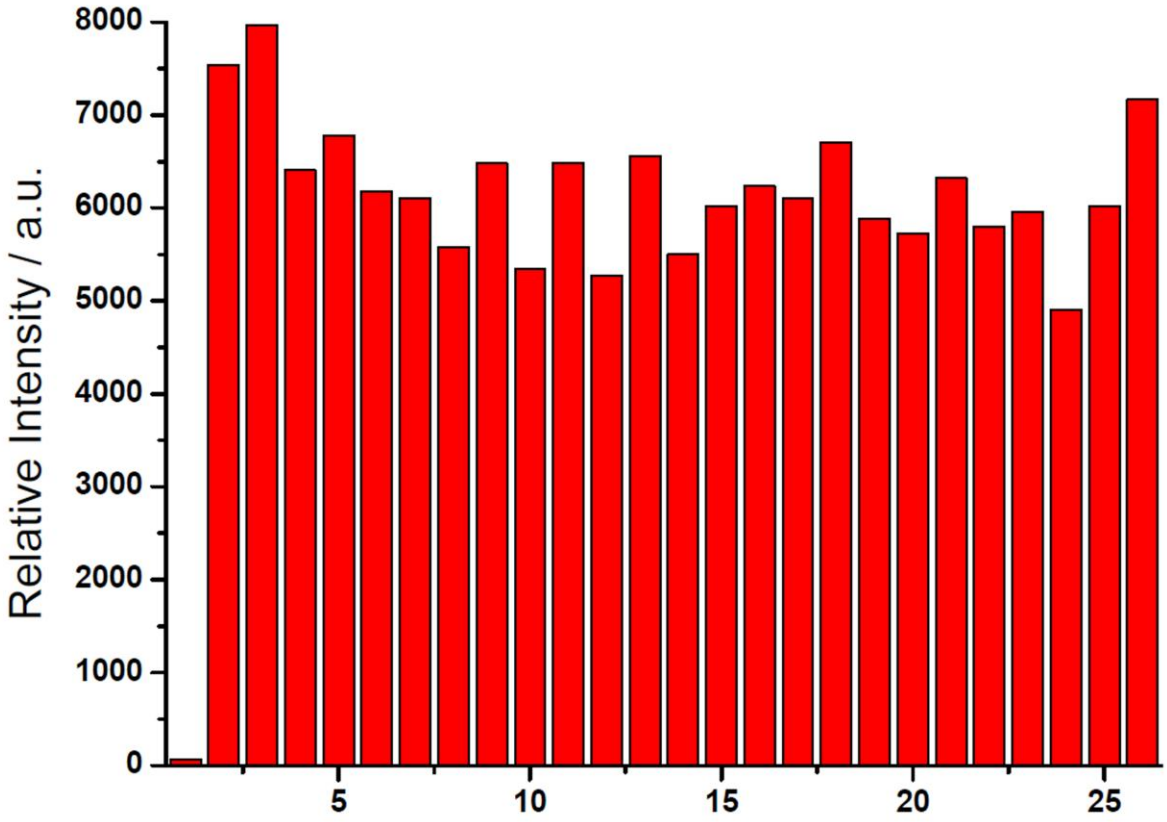


Fig. S6 Fluorescent intensities at 450 nm of **HOTA** (10 μM) in the presence of Cys (200 μM) and other bio-analytes (200 μM) in EtOH/PBS buffer solution (v/v, 1:1, pH 7.4): 1: **HOTA**, 2: **HOTA**+Cys, 3: 2+Hcy, 4: 2+GSH, 5: 2+NAC, 6: 2+Ala, 7: 2+*β*-ala, 8: 2+Arg, 9: 2+Asn, 10: 2+Asp, 11: 2+Gln, 12: 2+Glu, 13: 2+Gly, 14: 2+His, 15: 2+Ile, 16: 2+Lys, 17: 2+Leu, 18: 2+Mpa, 19: 2+Met, 20: 2+Pro, 21: 2+Phe, 22: 2+Ser, 23: 2+Tyr, 24: 2+Thr, 25: 2+Trp, 26: 2+Val. (λ_{ex} = 365 nm).

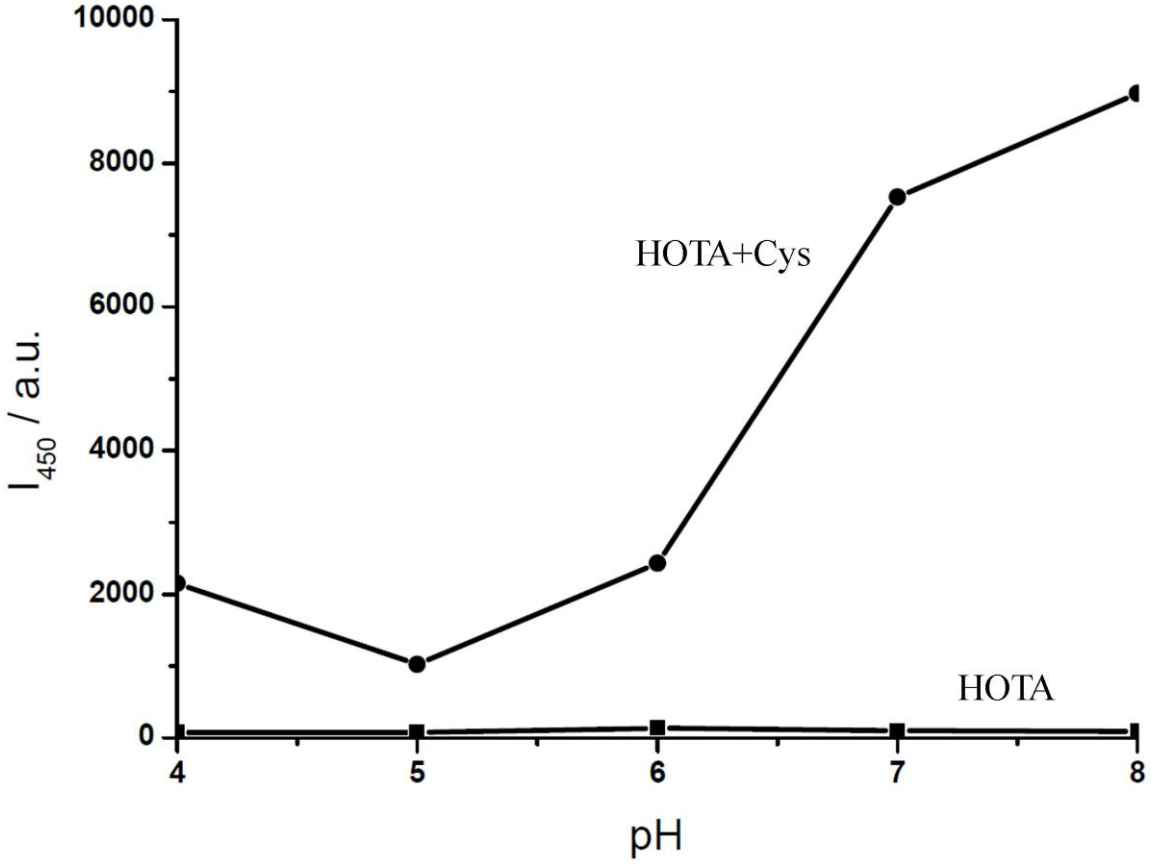


Fig. S7 Fluorescence intensity at 450 nm of **HOTA** (10 μM) and **HOTA** (10 μM) + Cys (200 μM) in EtOH/PBS buffer solution (v/v, 1:1) at different pH values. (λ_{ex} = 365 nm).

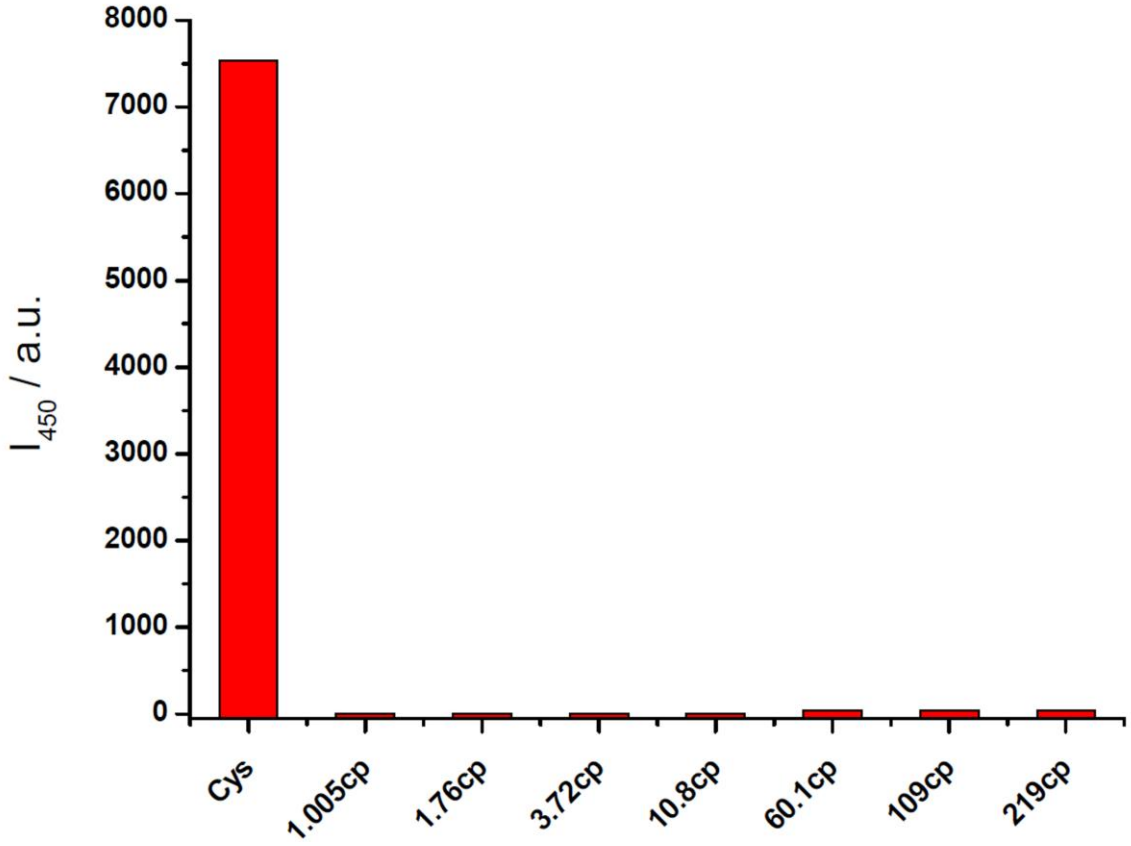
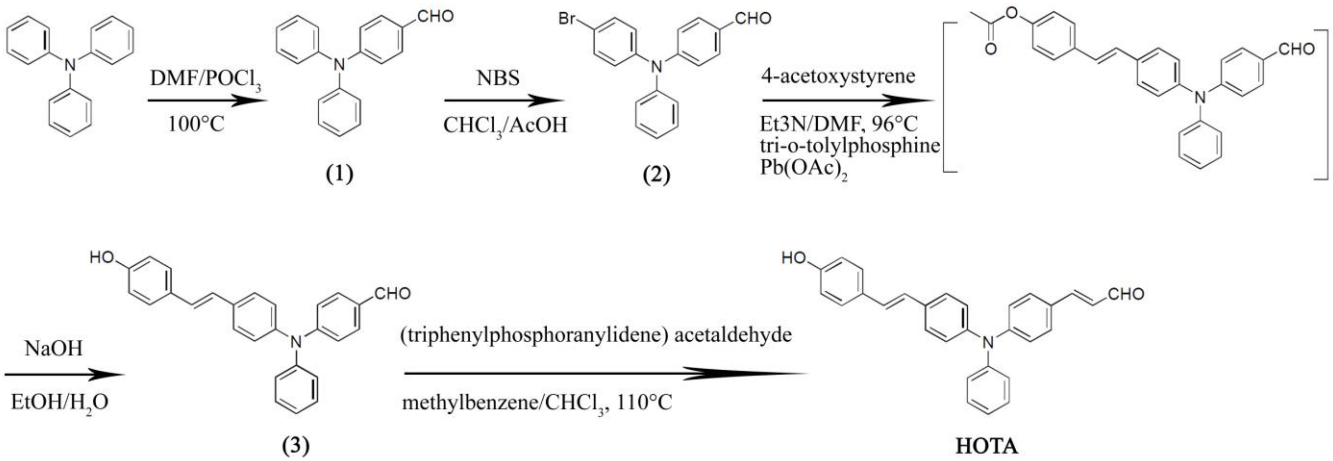


Fig. S8 Fluorescence intensity at 450 nm of **HOTA** (10 μM) + Cys (200 μM) in EtOH/PBS buffer solution (v/v, 1:1, pH 7.4) and **HOTA** (10 μM) in the solution of glycerol and H_2O with different viscosities.

Synthesis of HOTA



Scheme S1. The synthetic route of **HOTA.**

Synthesis of 4-formyltriphenylamine (1):

Phosphoryl chloride (2.02 mL, 24.5 mmol) was dropwisely added to a mixture of triphenylamine (5.0 g, 20.4 mmol) and dry dimethylformamide (35 mL) at 0 ℃ with stirring. The reaction mixture was stirred at room temperature for 1 h and then mixture was warmed at 100 ℃ under the protection of nitrogen for 8 h. After being cooled to room temperature, the reaction mixture was poured into ice-water, neutralized with NaOH solution and then extracted with CH_2Cl_2. The combined organic phase was washed with water and saturated brine, dried over anhydrous $MgSO_4$ overnight. After CH_2C1_2 was removed, the crude product was purified by column chromatography with ethyl acetate/petroleum ether (10:1) as eluent, finally the light-yellow solid was obtained for **1** with a yield of 83%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.77 (s, 1H), 7.72 (d, J = 8.7, 2H), 7.42 (t, J = 7.8, 2H), 7.24~7.20 (m, 6H), 6.88 (d, J = 8.7, 2H).

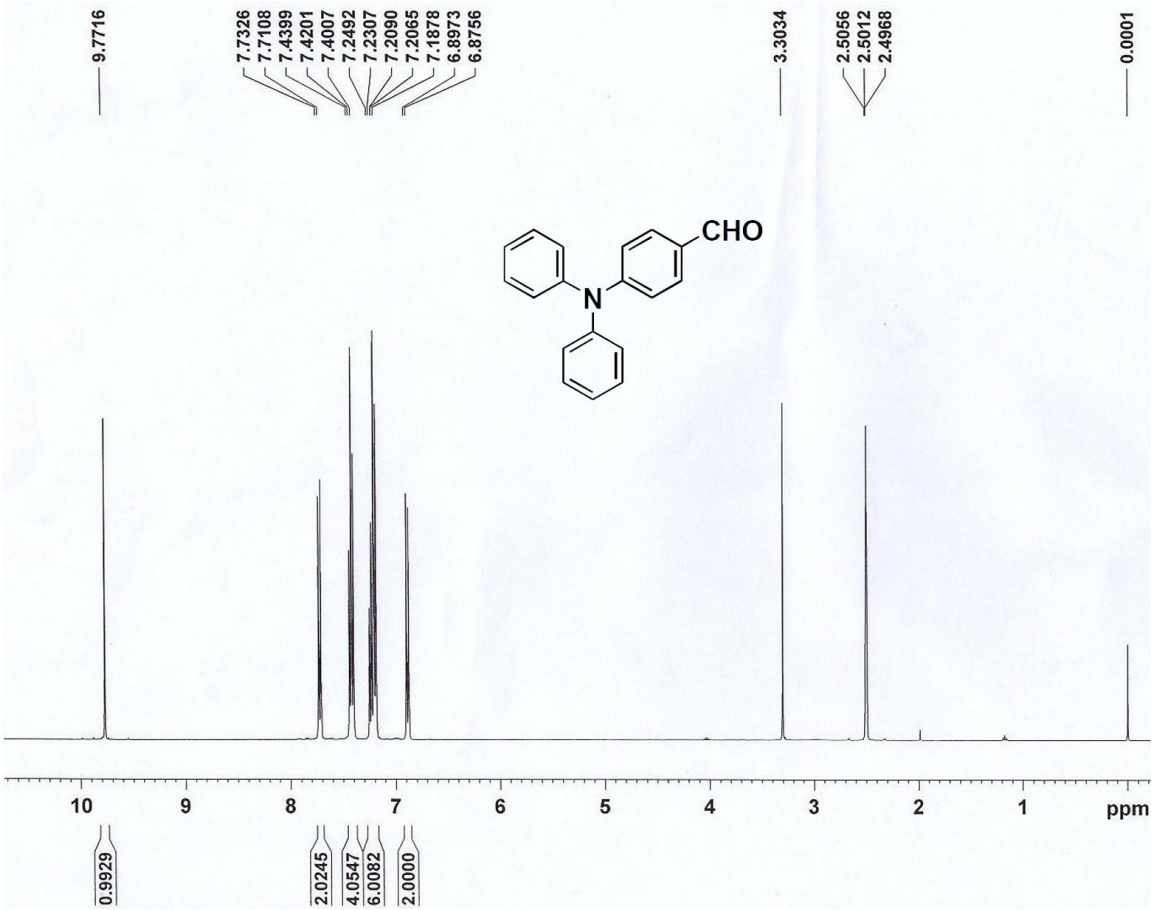


Fig. S9 ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

Synthesis of (4-bromophenyl)-(4-formylphenyl)-(phenyl)amine (2):

NBS (0.39 g, 2.20 mmol) was added to a solution of compound **1** (0.50 g, 1.83 mmol) in chloroform/AcOH (30 ml, v/v, 1:1) at room temperature. The mixture was stirred under the protection of nitrogen for 20 h at room temperature and then quenched with water, and the aqueous layer was extracted with CH_2Cl_2. The organic phase was washed with water and saturated brine, dried over anhydrous magnesium sulfate overnight. After CH_2C1_2 was removed, the crude product was purified by column chromatography with ethyl acetate/petroleum ether (10:1) as eluent, finally the light green solid was obtained for **2** with a yield of 86%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.80 (s, 1H), 7.75 (d, J = 8.60, 2H), 7.57 (d, J = 8.68, 2H), 7.43 (d, J = 7.76, 2H), 7.25 (d, J = 7.32, 1H), 7.19 (d, J = 7.88, 2H)7.12 (d, J = 8.64, 2H), 6.94 (d, J = 8.60).

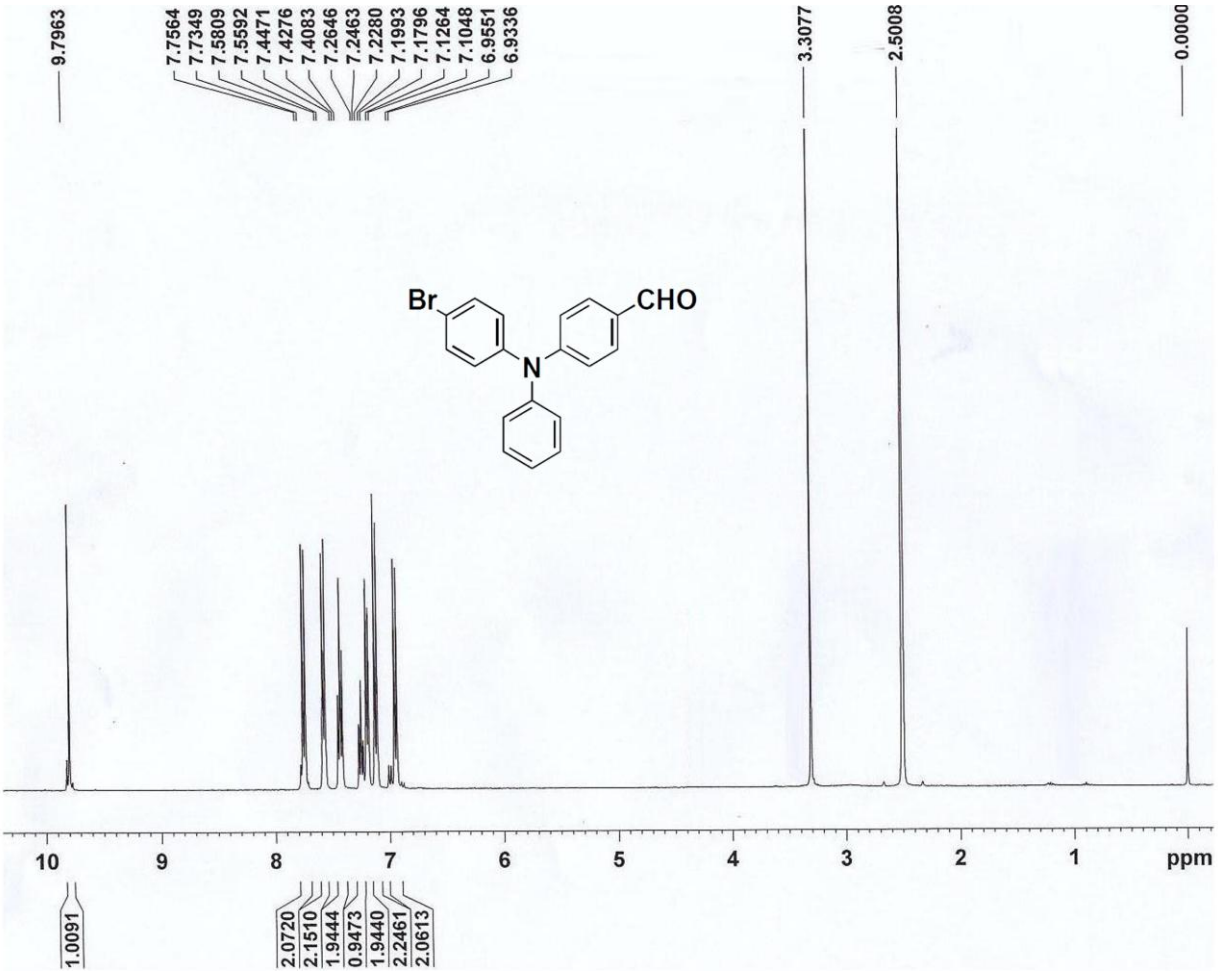


Fig. S10 ^{1}H NMR spectrum of compound **2** in DMSO-d_6.

Synthesis of (*E*)-(4-(4-hydroxystyrene)phenyl)-(4-formylphenyl)-(4-phenyl)amine (3):

Compound **2** (0.70 g, 1 mmol), palladium(II) acetate (22.5 mg, 0.1 mmol) and tri-(*o*-tolyl)phosphine (92.8 mg, 0.3 mmol) were introduced into a dry and dehydration TEA/DMF (21 mL, 2:1, v/v) mixture, which was stirred under the protection of nitrogen for 30 min. 4-acetoxystyrene (0.46 mL, 3.0 mmol) was added and then the reaction mixture was heated up to 96 ℃ under nitrogen atmosphere and kept at this temperature in an oil bath for 18 h. The resulting mixture was allowed to cool to room temperature. The crude mixture was diluted with dichloromethane, washed with water and saturated NaCl. After concentration, the remainder was directly transferred to a flask and mixed with EtOH/H_2O (40 mL, 1:1, v/v). Then solid NaOH (1 g, 0.025mol) was added to the system and stirred for 8 h. After that, 1 M HCl solution was continuously added until the pH value of the mixture was below 6. The solution was then

extracted with dichloromethane. The extraction liquid was distilled in vacuum and the residue was purified by column chromatography with ethyl acetate/petroleum ether (1:3) as eluent and yellow product (**3**) was obtained with a yield of 57%. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.78 (s, 1H), 9.60 (br, 1H), 7.74 (d, J = 8.60, 2H), 7.57 (d, J = 8.44, 2H), 7.43~7.40 (m, 4H), 7.2 (q, J = 7.81, 3H), 7.15~6.99 (m, 4H), 6.94 (d, J = 8.64, 2H), 6.77 (d, J = 8.44, 2H).

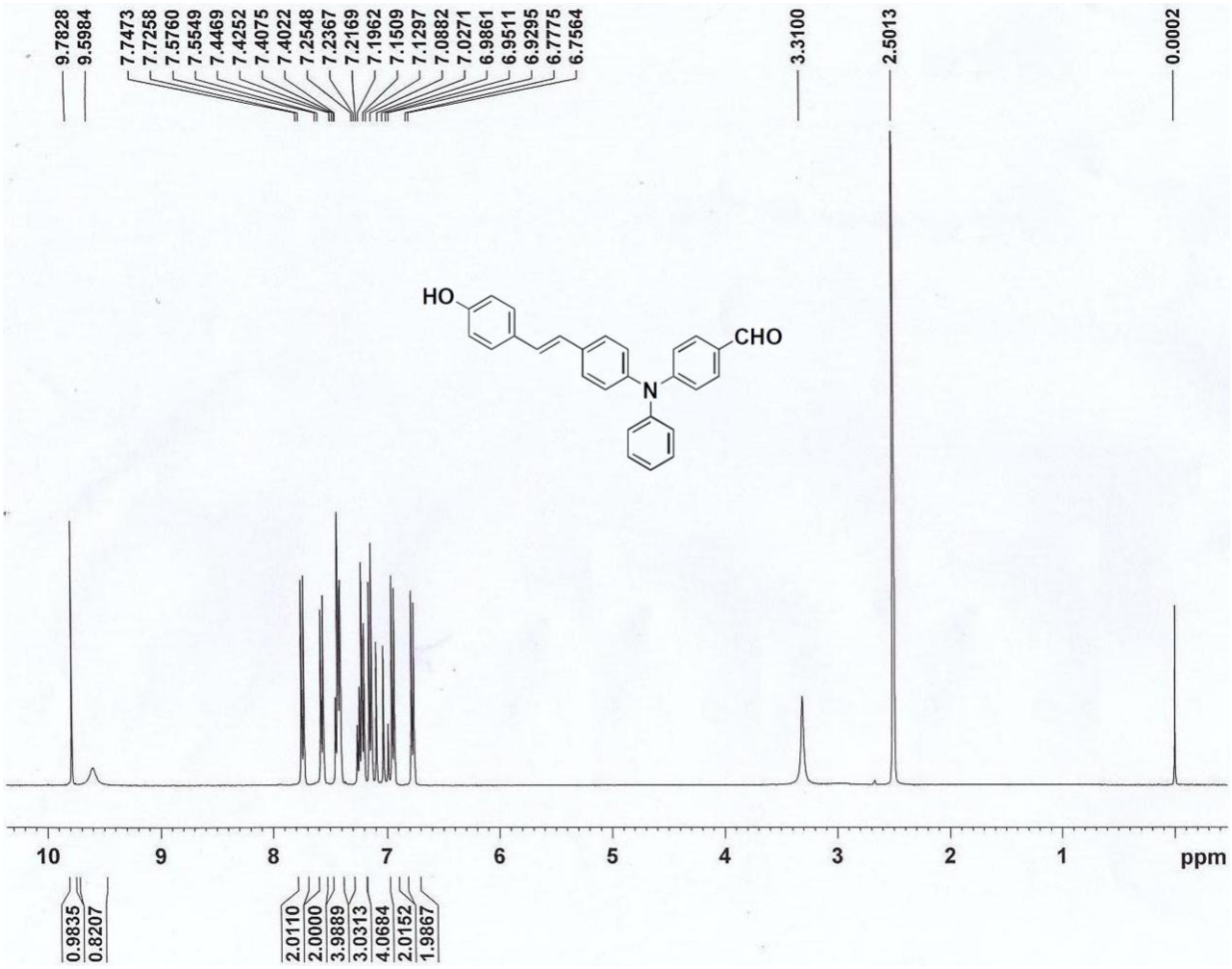


Fig. S11 ^{1}H NMR spectrum of compound **3** in DMSO-d_6.

Synthesis of (E)-(4-(4-cinnamoyl)phenyl)-(4-formylphenyl)-(4-phenyl)amine (HOTA):

Compound **3** (0.18 g, 2.0 mmol) was dissolved in 40 mL of 1,4-dimethylbenzene, then added into a flask and bubbled with nitrogen for 30 min. A solution of triphenylphosphoranylidene (182.62 mg, 0.6 mmol) in chloroform (10 ml) was added into the system. The mixture was then bubbled with nitrogen for 30 min and heated at 110 ℃ for 24 h under the protection of nitrogen and a brownish red suspension was obtained. The mixture was distilled to remove solvent, then poured into H_2O (500 mL) and

extracted with CH_2Cl_2 after the resulting mixture was cooled to room temperature. The organic phase was separated, dried with $MgSO_4$ and removed by vacuum distillation. Red powder product was obtained after the residue was purified by column chromatography with ethyl acetate/petroleum ether (1:4, v/v) as eluent with a yield of 36.5%. ^{1}H NMR (400 MHz, DMSO): δ (ppm) 9.60 (d, J = 7.88, 1H), 9.55 (s, 1H), 7.63 (t, J = 7.98, 3H), 7.53 (d, J = 8.60, 2H), 7.40 (m, 4H), 7.20~6.92 (m, 9H), 6.76 (d, J = 8.52, 2H), 6.69 (dd, J_1 = J_2 = 7.88, 1H). ^{13}C NMR (300 MHz, DMSO-d_6), δ (ppm) =194.41, 157.73, 153.30, 150.37, 146.46, 145.30, 134.35, 130.75, 130.33, 128.68, 128.50, 128.24, 127.83, 127.37, 126.54, 126.03, 125.80, 125.16, 124.87, 121.03, 116.04. HRMS (m/z): calcd for $C_{27}H_{23}NO_2$: 417.17; found: m/z 418.18 $(M + H)^+$. IR (cm^{-1}): 1651 ($\upsilon_{C=O}$).

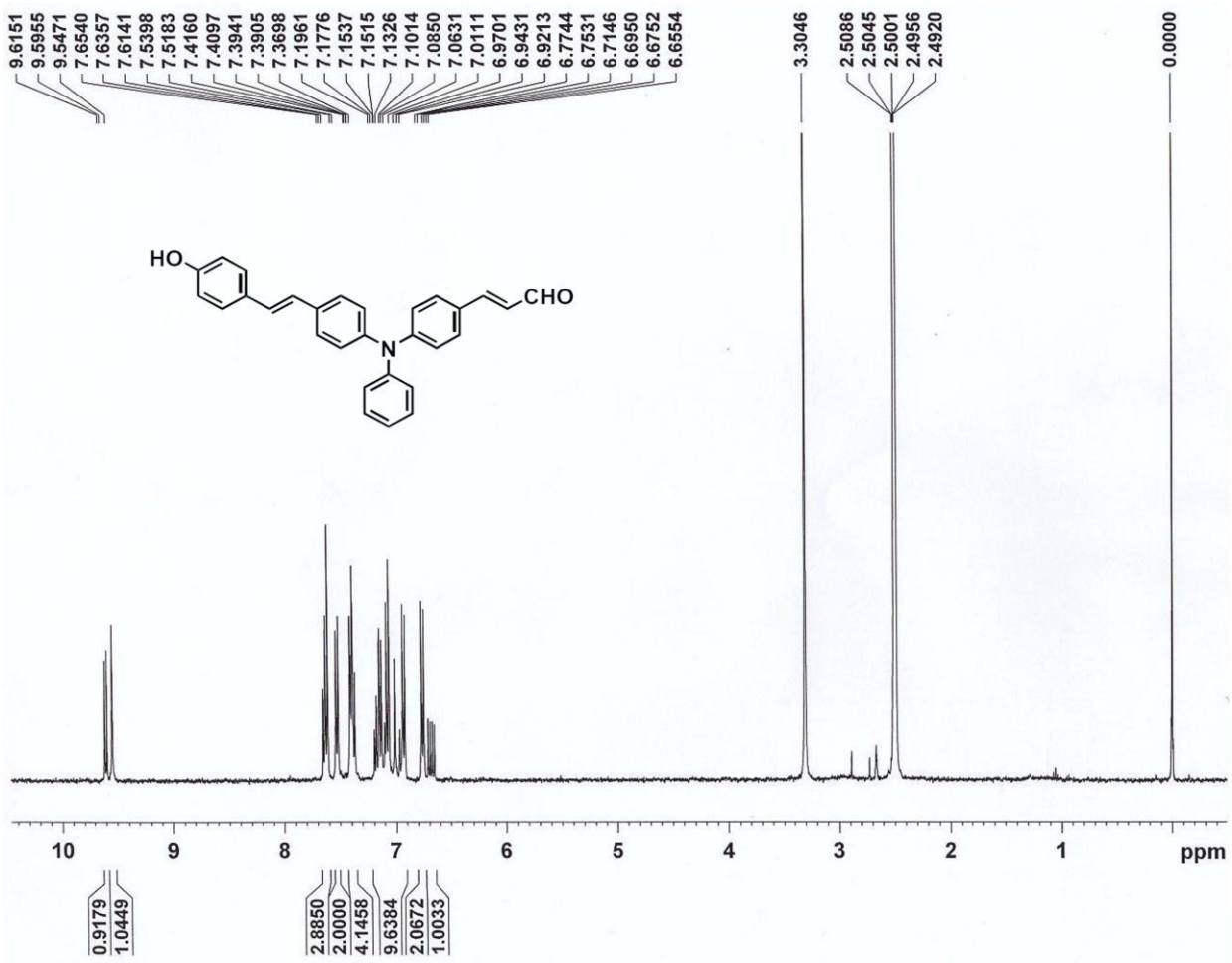


Fig. S12 ^{1}H NMR spectrum of **HOTA** in DMSO-d_6.

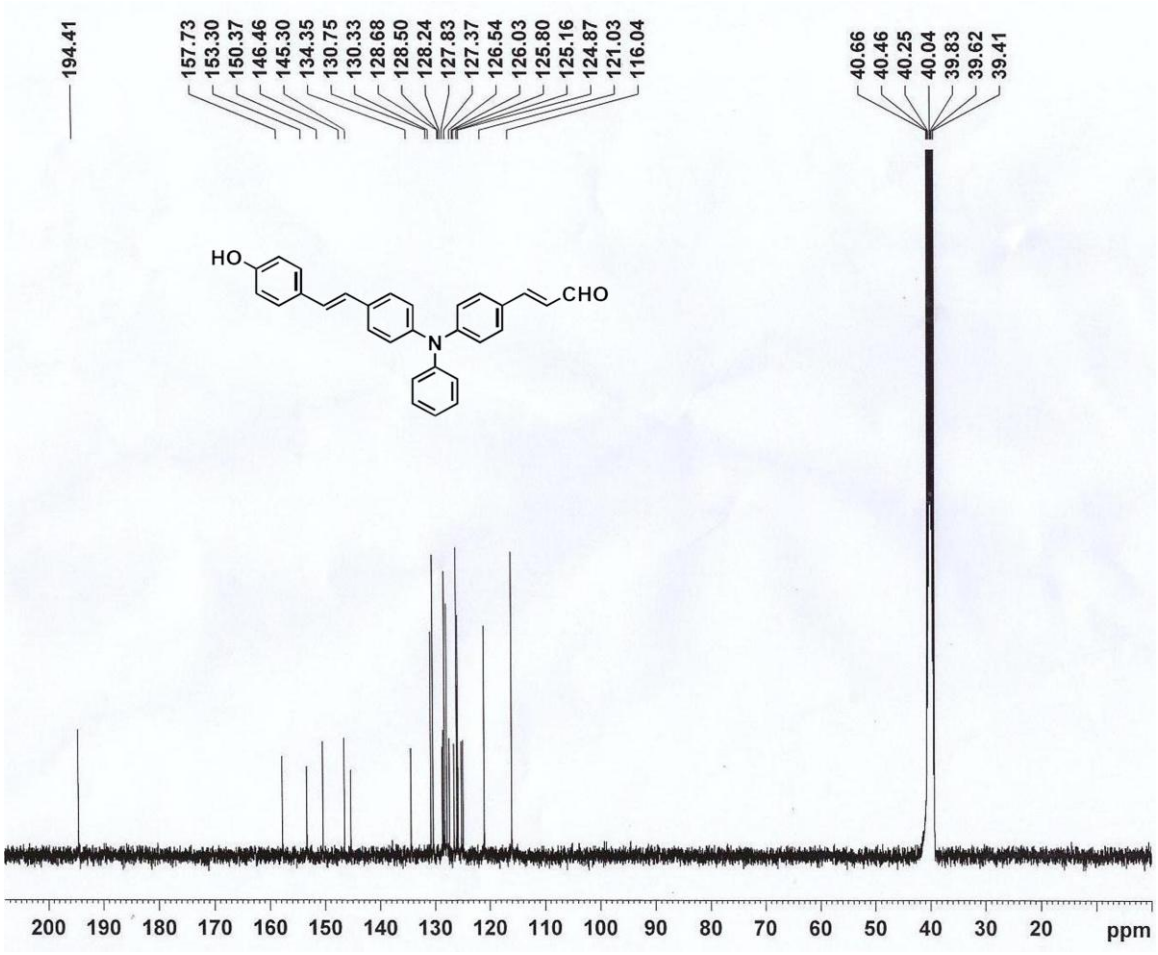


Fig. S13 ^{13}C NMR spectrum of **HOTA** in DMSO-d_6.

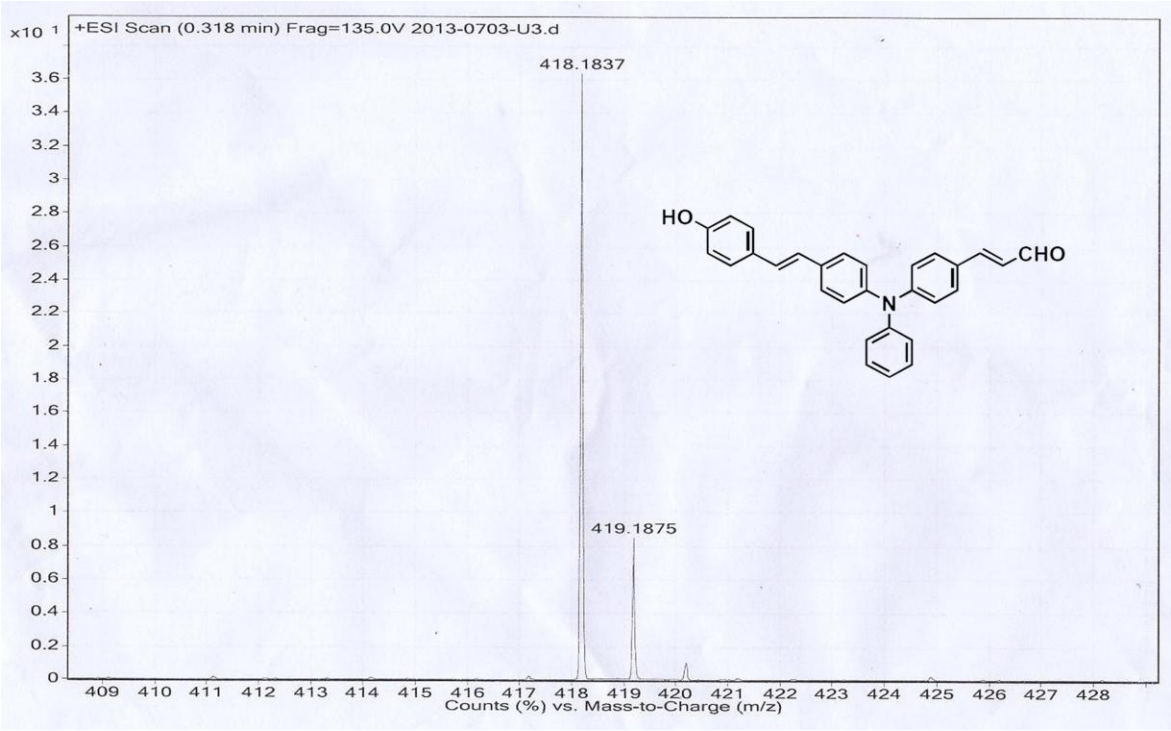


Fig. S14 HRMS of **HOTA** in EtOH.

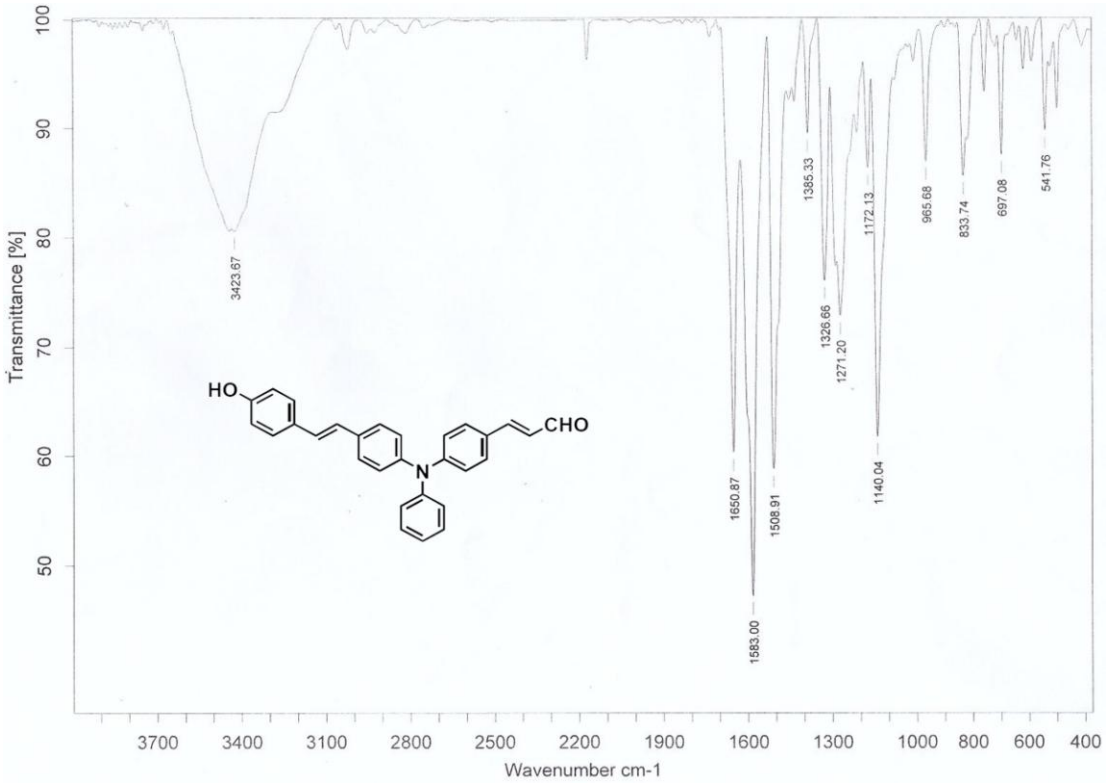


Fig. S15 IR spectrum of **HOTA.**