A novel luminol-related hydroxyphenyl benzothiazole analogue chemiluminescence probe with ultrahigh sensitivity for bloodstain detection

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Experimental section

Synthesis of 4-amino-5-mercaptophthalhydrazide (AMPH) and 2-(4-(dimethylamino)phenyl)-6,7-dihydrothiazolo[4,5-g]phthalazine-5,8-dione (DAP-AMPH)

The synthesis of AMPH was according to the previous literature.[S1] First, the 4-amino-5-mercaptophthalimide (AMP) was synthesized. The 4-Aminophthalimide (4 g, 27.19 mmol), NaSCN (4642 mmol) and methanol (62 mL) were added in a three-mouth flask placed in ice water bath, then cooled to 0°C. The liquid bromine (3.2 mL, 64 mmol) was dissolved in methanol (31 mL) and placed in a drip funnel. When the temperature of the solution in the flask reached -5°C, the methanol solution of bromine was added dropwise. The solution was kept at -5°C for 2 h post-dropping and at room temperature for another 8 h. After that, the solution was filtrated and the filter cake was recrystallized with methanol. Through suction filtration and vacuum drying, a light-yellow solid powder was obtained, namely AMP. Secondly, the AMP (0.7928 g, 4.03 mmol) was dissolved in ethanol (17 mL). This solution was stirred and kept at a constant temperature of 56°C. Hydrazine hydrate (4 mL, mass fraction 85%) was dropwise added to the solution for 2 h. After that, the solution was refluxed for 48 h. At the end of the reaction, ethanol-water was used as a solvent for reduced pressure distillation, and recrystallized with absolute ethanol was conducted to obtain a grey powder product AMPH.

For synthesis of DAP-AMPH, AMPH (0.21 g, 1 mmol) and 4-dimethylaminobenzaldehyde (0.18 g, 1 mmol) were dissolved in DMF (20 ml), sodium hydrogen sulfite (0.75 g, 7.2 mmol) and phosphorous acid (6.25 g, 76.2 mmol) were added to sulfuric acid (20 ml, 1.5 M), and the mixed solution was added to the reaction solution. Stir and heat at 60 °C for 2 h, then stir for 3 h at room temperature. Cooled in ice water, and filtered to form a precipitate, methanol recrystallization, DAP-AMPH (orange crystal) are obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 7.60 (d, J = 8.4 Hz, 2H), 6.72 (d, J = 8.5 Hz, 2H), 2.95 (d, J = 2.0 Hz, 6H).

Synthesis of Urea hydrogen peroxide (UHP)

According to the literature,[S2] urea (15 g, 0.25 mol), hydrogen peroxide (0.3 mol, mass fraction 30%), stabilizer NaH_2PO_4 and coating agent PE-400 were added in the 250 mL three-mouth flask.

and then the solution was stirred in the constant temperature water bath at 30 °C for 50 min. After that, the solution was crystallized, filtered, and dried at -5 °C to obtain UHP.

Preparation of solutions

For detection of properties of DAH-AMPH:

pH-B-R buffer solution: in 100 mL of triacid mixture (phosphoric acid, acetic acid, boric acid, concentration of 0.04 M of each), different volumes of 0.2 M NaOH were added to obtain the pH-B-R buffer solutions with certain pH values.

DAH-AMPH solution 1: 3.6 mg of DAH-AMPH was dissolved in 20 mL of DMSO forming a 500 μM stock solution and stored at -20°C without light.

For hemin detection:

pH buffer solution (pH 12.5): 200 mM NaH_2PO_4 and NaOH aqueous solution were mixed evenly according to the volume ratio of 1:3 to obtain the pH buffer solution with 12.5 of pH values.

UHP solution 1: 10 mM UHP was diluted to 30 mM by pH buffer solution (pH 12.5).

DAH-AMPH solution 2: DAH-AMPH was dissolved in NaOH solution to obtain a 3 mM stock solution, diluted with 25 mM NaOH as a solvent to 90 μM working solution for hemin detection.

Hemin solution: hemin was dissolved in DMSO to obtain a 10 mM stock solution. The pH buffer solution (pH 12.5) was used to dilute the stock solution forming a 30 μM working solution.

For HRP measurement assay:

pH buffer solution (pH 8.0): 12.1 g Tris was dissolved in 80 mL ultrapure water and added in 4.2 mL HCl, then the solution volume was set to 1000 mL forming a 1 M Tris-HCl buffer solution. This solution was diluted to 50 mM for reserve with a pH of 8.0.

DAH-AMPH solution 3: DAH-AMPH was dissolved in DMSO to obtain a 100 mM stock solution. This stock solution was diluted to 7.5 mM by pH buffer solution (pH 8.0).

UHP solution 2: HRP was dissolved in ultrapure water to obtain a 100 mM stock solution, and adjust pH=3~4 to ensure stability. pH buffer solution (pH 8.0) was applied to dilute the stock solution to 10 mM of concentration.

HRP solution: The HRP solid powder was dissolved in pH buffer solution (pH 8.0) (containing 1 mg/mL BSA) to get a 10 mg/mL stock solution, then it was diluted to vary concentrations.

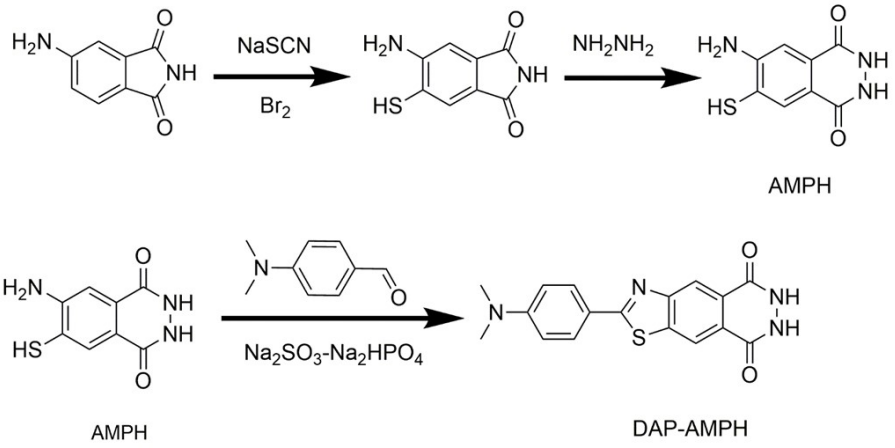

Figure S1. Synthesis routes of AMPH and DAP-AMPH.

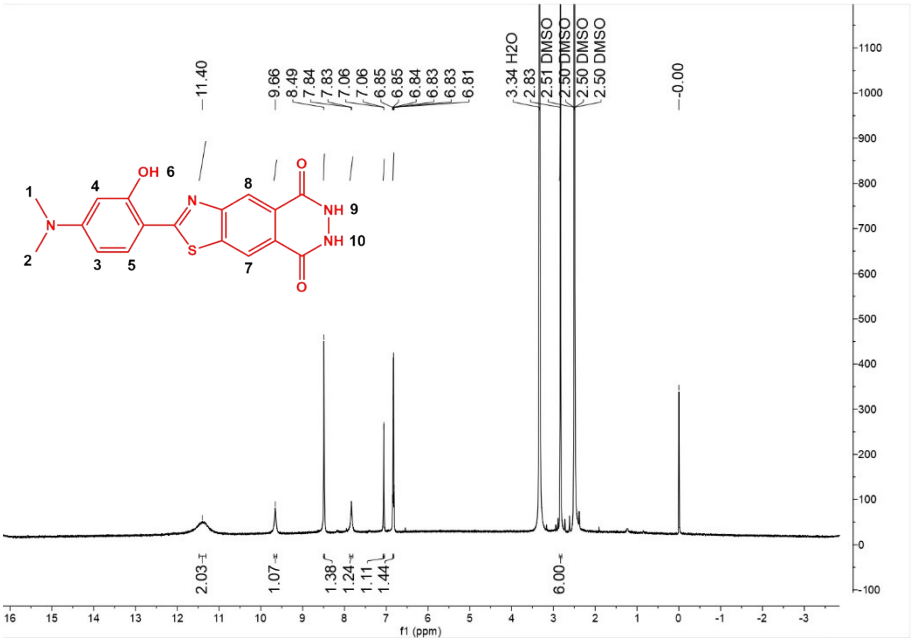

Figure S2. ^{1}H NMR spectra of DAH-AMPH.

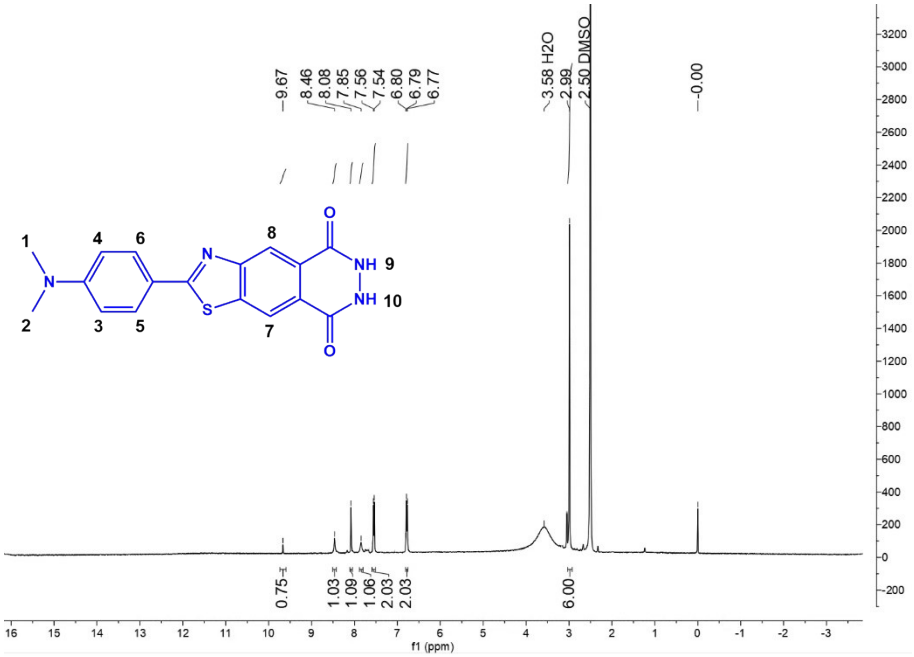

Figure S3. ^{1}H NMR spectra of DAP-AMPH

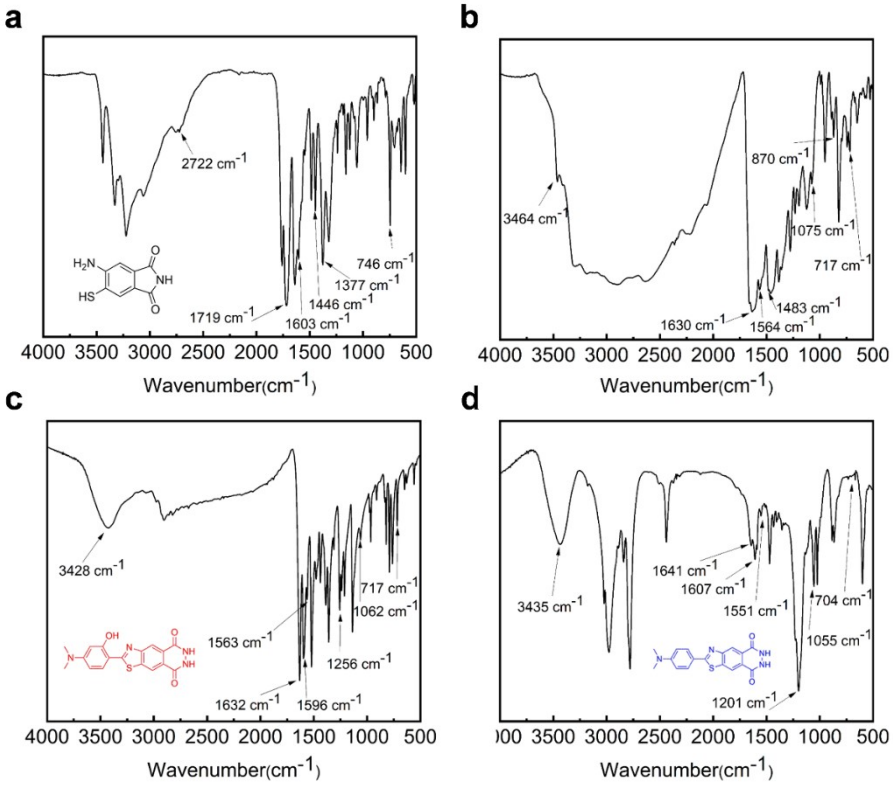

Figure S4. The IR spectra of AMP (a), AMPH (b), DAH-AMPH (c), and DAP-AMPH (d).

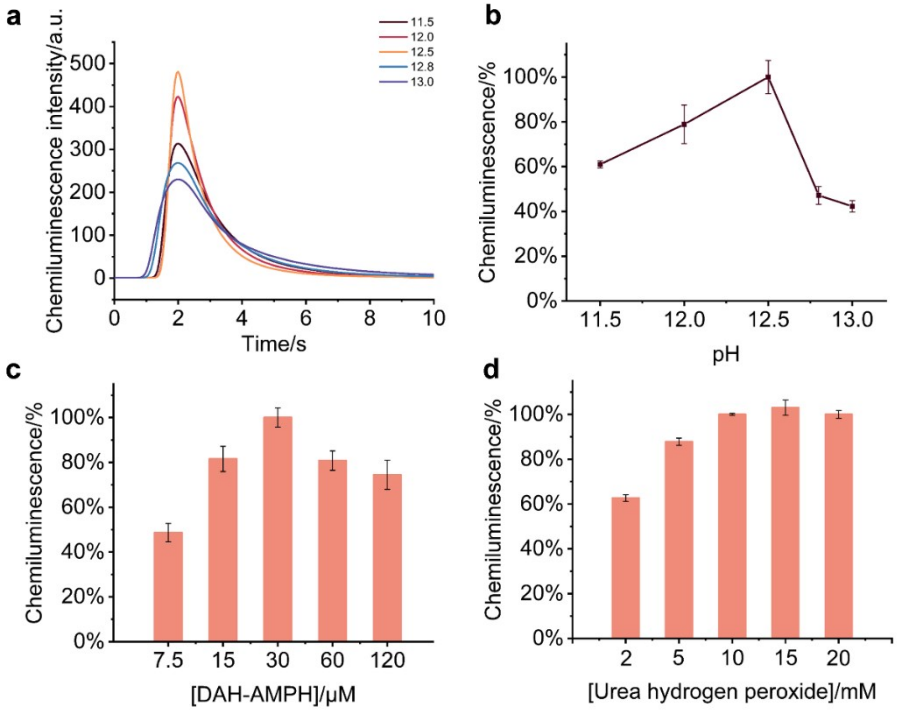

Figure S5. Optimization for pH condition and concentrations of DAH-AMPH and UHP. (a) CL intensity-time curves at different pH values. (b) Relationship of the CL peak intensity and pH values. Concentrations: DAH-AMPH 0.03 mM, hemin 10 μM, and UHP 10 mM. Dependence of CL peak intensities on the concentrations of DAH-AMPH (c) and UHP (d). Hemin is 10 μM and pH is 12.5. CL intensity was recorded 100 s after the start of the reaction. Each point represents the mean of triplicate experiments. Vertical bars indicate ±SD about the mean (n = 3).

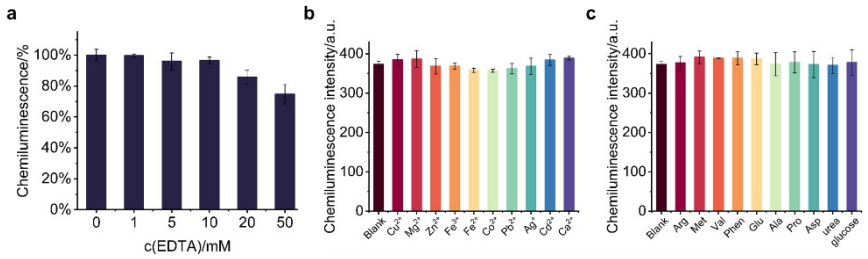

Figure S6. (a) Effect of EDTA on the CL peak intensity. The concentrations were 0.03 mM of DAH-AMPH, 10 μM of hemin, 10 mM of UHP, and 12.5 of pH value. Selectivity of DAH-AMPH against common metal ions (b) and biomolecules (c). Blank represents the CL system of DAH-AMPH/ UHP /hemin only. The concentration of all the metal ions was 0.1 mM, and the concentration of all the biomolecules was 1.0 mM. The concentrations of reaction components in the DAH-AMPH/ UHP /hemin CL system were 0.03 mM of DAH-AMPH, 10 μM of hemin, 10 mM of UHP, and 12.5 of pH value. Vertical bars indicate ±SD about the mean (n = 3).

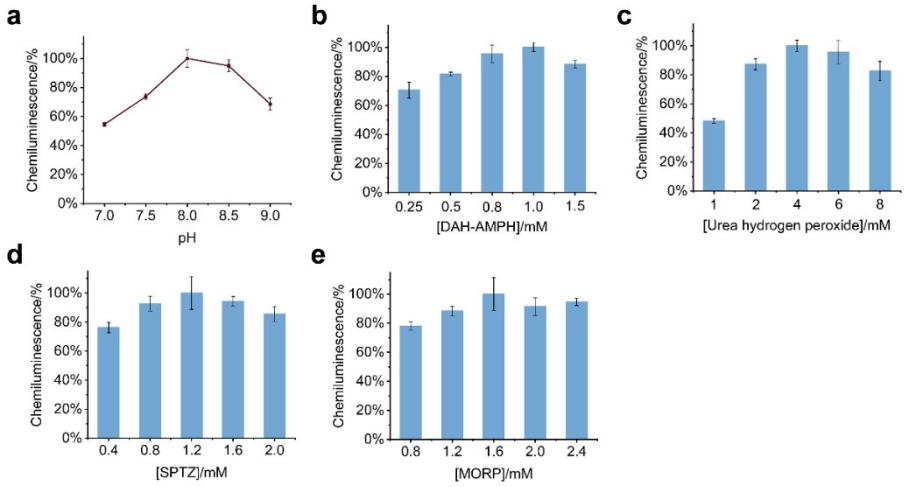

Figure S7. Optimization of pH condition and concentration of reactants. (a) The CL intensity ratios between HRP and background in DAH-AMPH/UHP/SPTZ/MORP/HRP CL system at different pH values. The concentrations were 250 pg/well of HRP, 1 mM of DAH-AMPH, 4 mM of UHP, 1.2 mM of SPTZ, and 1.6 mM of MORP. The CL intensity ratios of DAH-AMPH (b), UHP (c), SPTZ (d), and MORP (e) to background with various concentrations. HRP is 250 pg/well and pH is 8.0.

CL intensity was recorded 100 s after the start of the reaction. Vertical bars indicate ±SD about the mean (n = 3).

Table S1 Comparison of different methods for hemin detection.

Methods	Probes	Linear range	LOD	Ref.
Fluorescence	CdS QDs/protamine	0.167-17 μM	48.6 nM	S3
Electrochemilumine scence	Lucigenin/H_2O_2	0.015-15 μM	15 nM	S4
Fluorescence	Artemisinin-thiamine	2.0-300.0 nM	0.68 nM	S5
Fluorescence	GSH-Au NCs	1-25 nM	0.43 nM	S6
Chemiluminescence	Luminol/artemisinin	1-100 nM and 0.2-10.0 μM	0.37 nM	S7
Chemiluminescence	Luminol/artesunate	0.8-1000.0 nM	0.22 nM	S8
Chemiluminescence	DAH-AMPH/urea hydrogen peroxide	0.1-20 nM	2 pM	This work

Reference

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