Supporting Information (SI)

In situ generated chromophore as indicator for background-free sensing strategy of hydrazine and application in *vitro* and *vivo*

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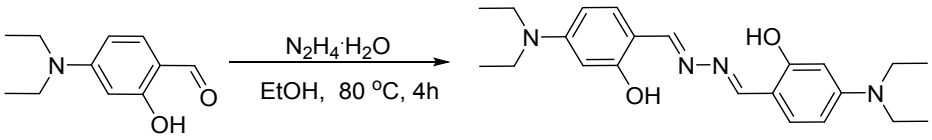
Contents

1. Synthesis of HDBM



Scheme S1. Synthesis route of HDBM.

0.58 g (3.0 mmol) 4-diethylaminobenzaldehyde and 0.50 mL (8.5 mmol) hydrazine hydrate (85%) were stirred into 20 ml EtOH and several drops of glacial acetic acid were added as catalyst. The contents were heated at 80 °C with stirring for 4 h after which the solution was distilled off in vacuo and the resulting residue was recrystallized from EtOH/diethyl ether furnished the pure product (0.46 g) in 82% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 11.47 (s, 2H), 8.59 (s, 2H), 7.26 (d, J = 8.8 Hz, 2H), 6.30-6.28 (m, 2H), 6.10 (d, J = 1.7 Hz, 2H), 3.36 (q, J = 6.9 Hz, 4H), 2.48 (t, J = 7.0 Hz, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm) ^{13}C NMR (126 MHz, DMSO) δ 161.08, 133.44, 106.85, 104.49, 97.50, 44.29, 12.99. ESI-MS *m/z*: $[M]^+$ Calcd for $C_{22}H_{31}N_4O_2^+$ 382.2369; Found 382.2376.

2. ^{1}H and ^{13}C NMR spectra

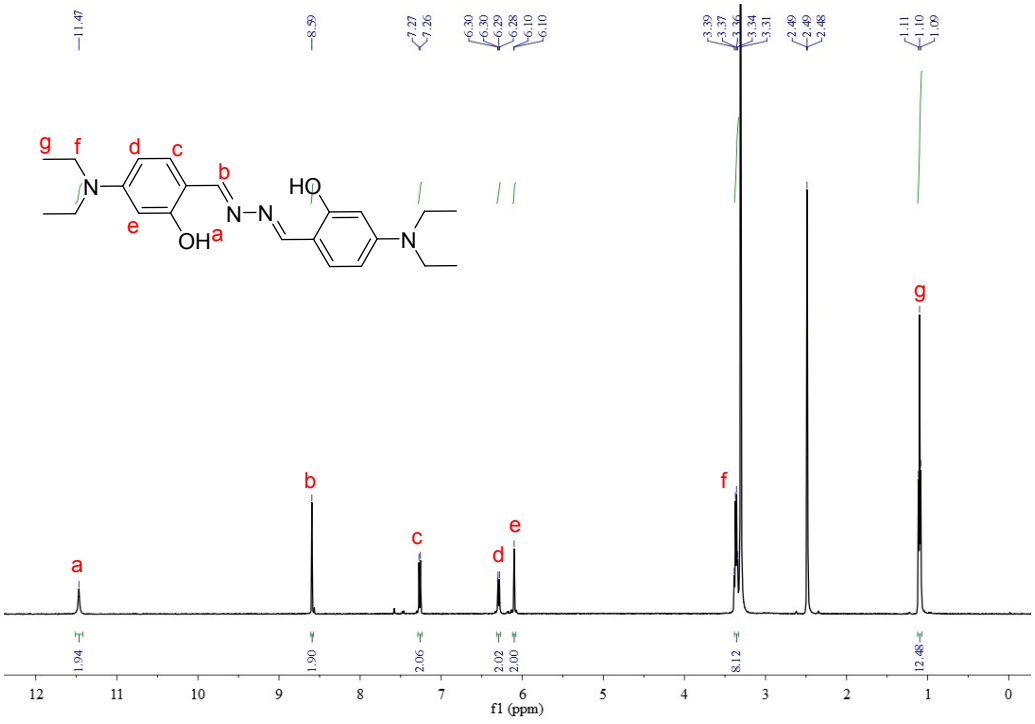


Figure S1 ^{1}H NMR spectrum of HDBM in DMSO-d_6.

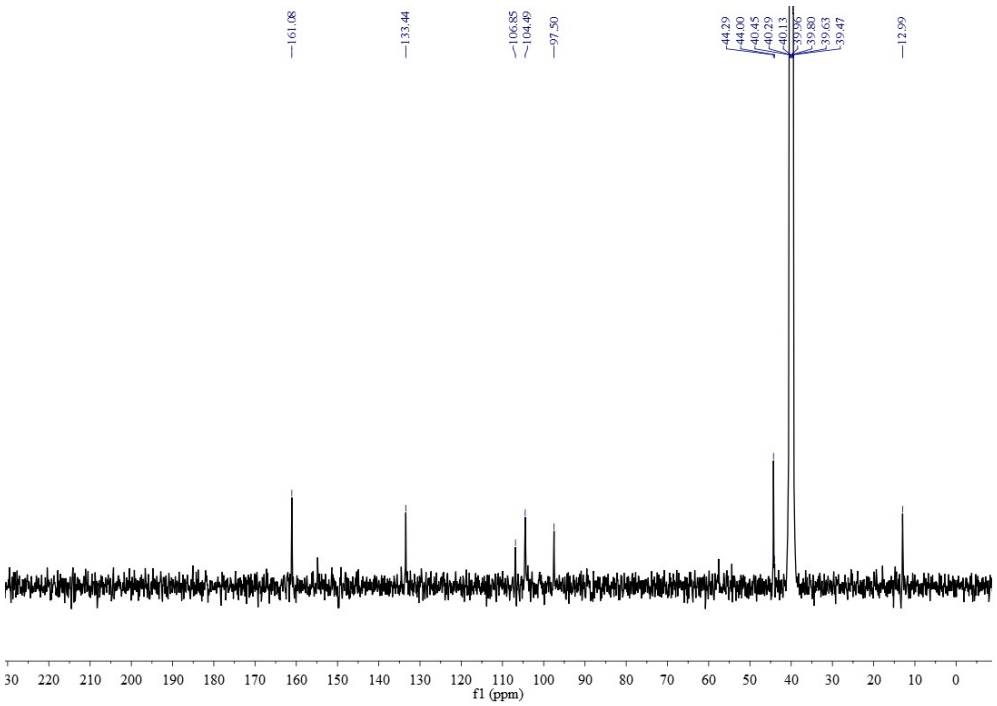


Figure S2 ^{13}C NMR spectrum of HDBM in DMSO-d_6.

3. Spectral Figures

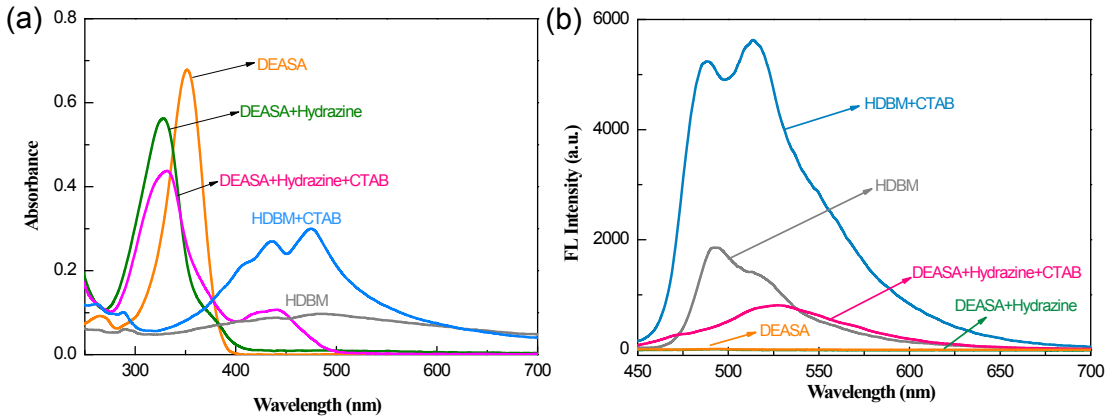


Figure S3 (a) Absorption and (b) fluorescence spectra of DEASA/HDBM in the presence or absence of hydrazine and CTAB. [DEASA] = 20 μM, [hydrazine] = 1.0 mM, [CTAB] = 1 mM, [HDBM] = 10 μM, λ_{ex} = 420 nm.

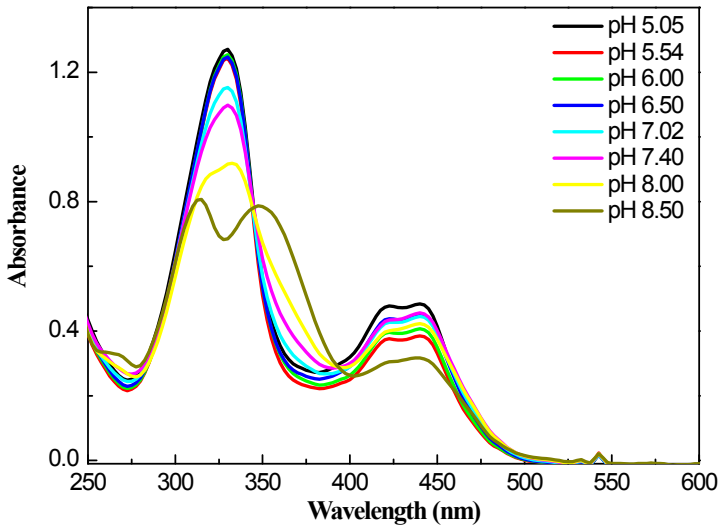


Figure S4 Absorption spectra of DEASA and hydrazine reaction solution containing CTAB in PBS buffer of varying pH. [DEASA] = 50 μM, [Hydrazine] = 2.5 mM, [CTAB] = 1mM.

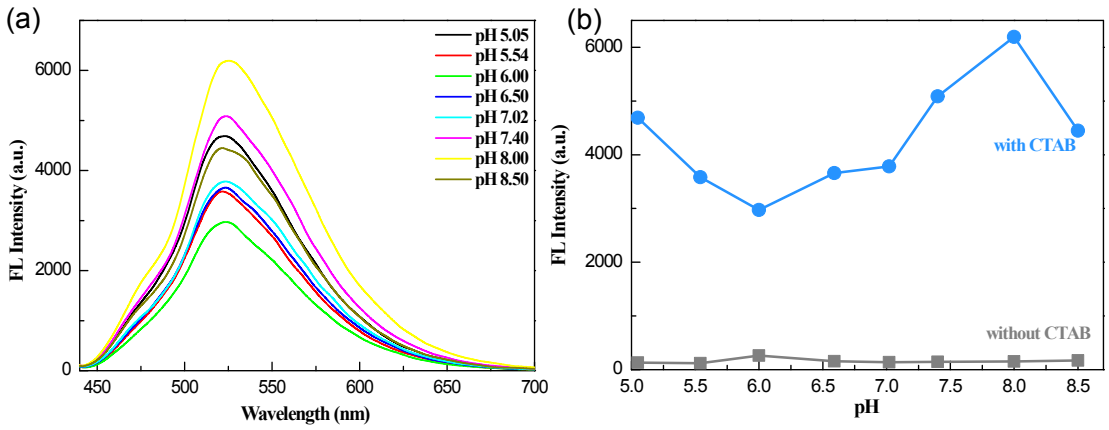


Figure S5 (a) Fluorescence spectra of DEASA and hydrazine reaction solution containing CTAB in PBS buffer of varying pH. (b) Plots of intensity at 525 nm after reaction of DEASA and hydrazine in the presence (red dots) or absence (black squares) of 1.0 mM CTAB. [DEASA] = 50 μM, [Hydrazine] = 500 μM, λ_{ex} = 420 nm.

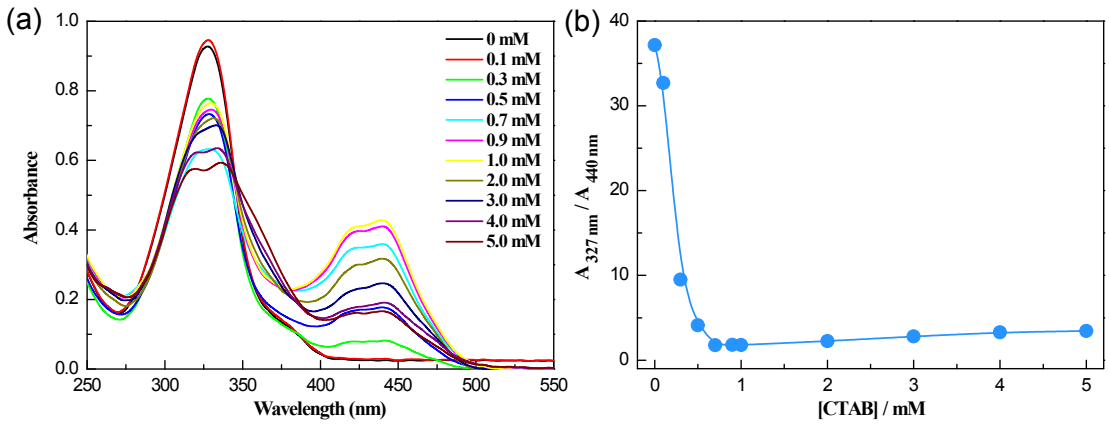


Figure S6 (a) Absorption spectra of DEASA and hydrazine reaction solution in PBS buffer (10 mM, pH 7.4) containing CTAB of varying concentrations. (b) Plots of absorption ratio ($A_{327\ nm}/A_{440\ nm}$) versus CTAB concentrations. [DEASA] = 50 μM, [hydrazine] = 500 μM.

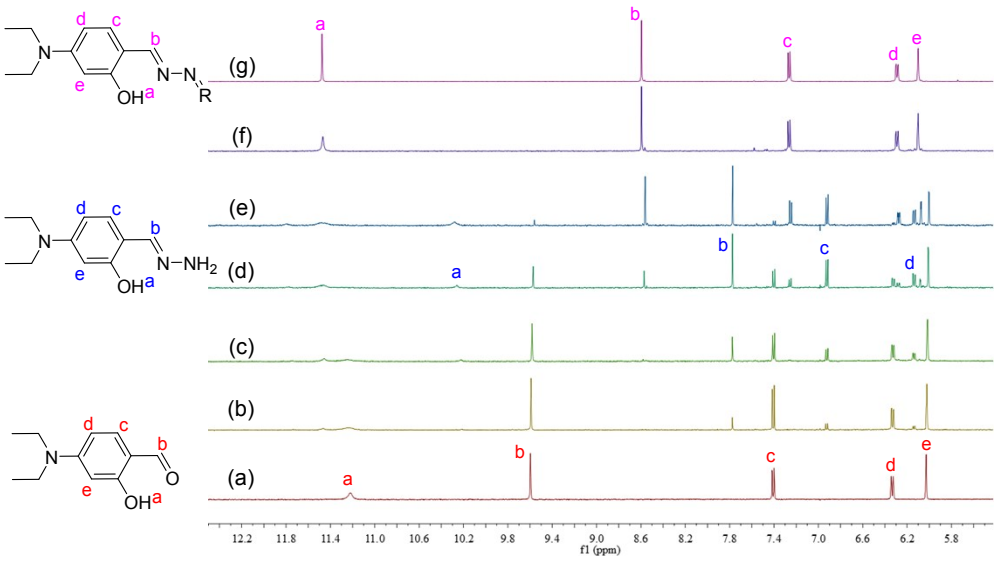


Figure S7 ^{1}H NMR spectra of (g) HDBM and DEASA (a) (2.5 mM, DMSO-d_6) with the addition of hydrazine (DMSO-d_6 solution) of (b) 0.25 mM, (c) 0.5 mM, (d) 1.25 mM, (e) 2.5 mM and (f) 12.5 mM.

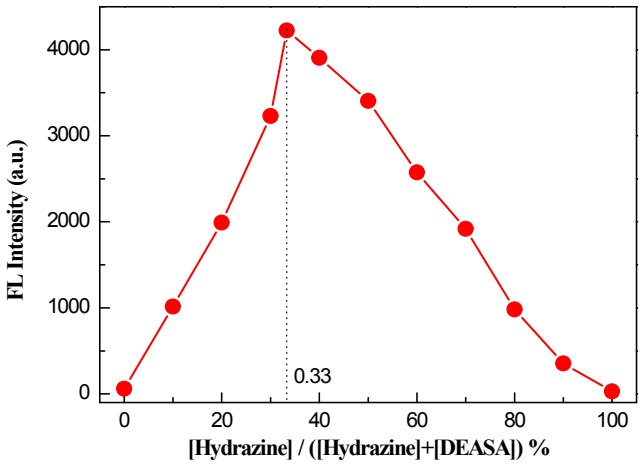


Figure S8 Job plot of fluorescence at 525 nm against [hydrazine]/([hydrazine]+[DEASA]). Total concentration of hydrazine and DEASA was 100 μM.

4. Cell Cytotoxicity

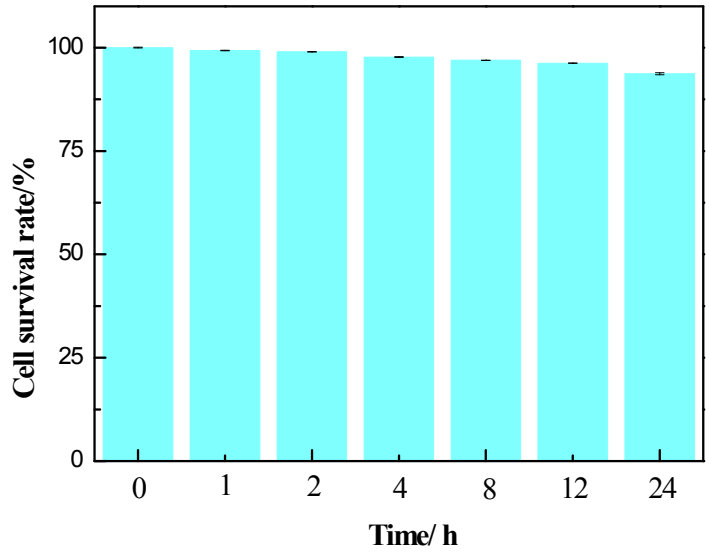


Figure S9 Cytotoxicity of DEASA against HeLa cells as determined by CCK-8 assay: HeLa cells were treated with 50 μM DEASA for 0-24 hours.

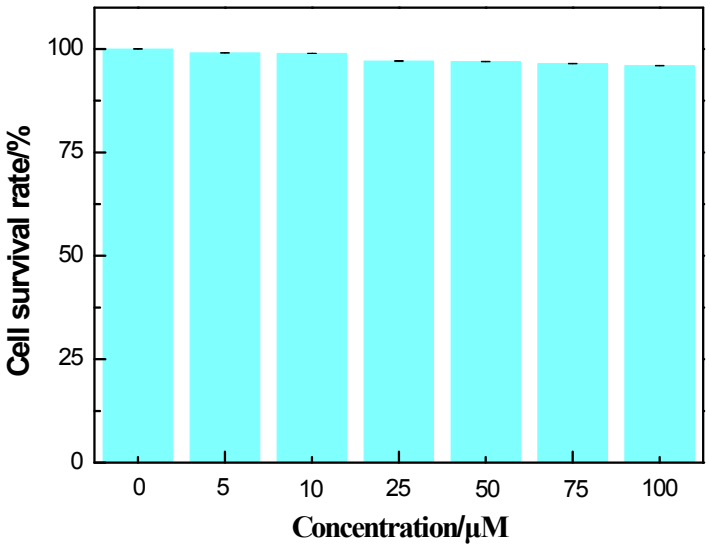


Figure S10 Cytotoxicity of DEASA against HeLa cells as determined by CCK-8 assay: HeLa cells were treated with DEASA (0-100 μM) for 2 hours.