Supplementary Information for

A simple design of fluorescent probes for indirect detection of β-lactamase based on AIE and ESIPT processes

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Content

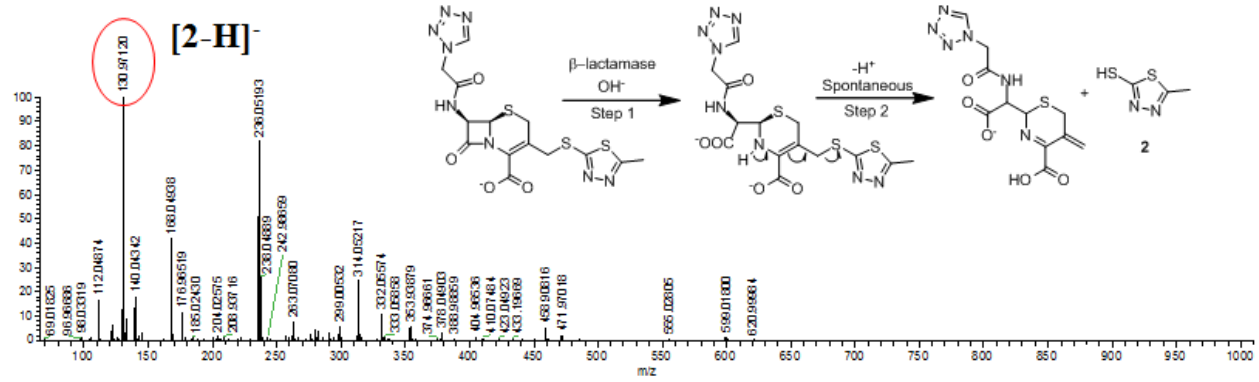

Fig. S1 ESI mass spectrum of cefazolin sodium (2.4 mM) upon addition of β-lactamase (1.0 U/mL) for 30 min in PBS buffer solution (10 mM, pH 7.4, 37 ℃), suggesting the formation of the thiol containing product **2.**

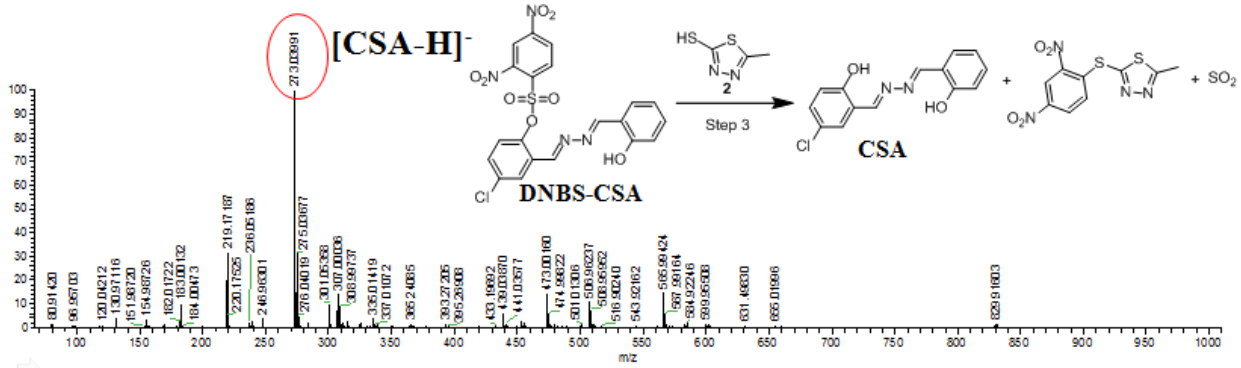

Fig. S2 ESI mass spectrum of the isolated fluorescent product after **DNBS-CSA** (2.0 mM) reacted with compound **2** (2.4 mM) for 30 min in PBS buffer solution (10 mM, pH 7.4, 37 ℃), suggesting the formation of **CSA** from **DNBS-CSA.**

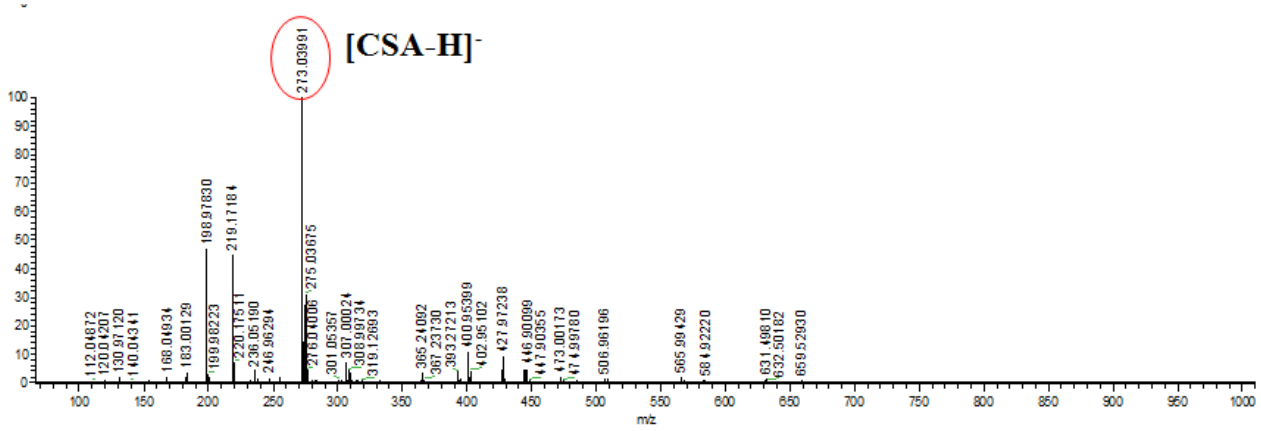

Fig. S3 ESI mass spectrum of the isolated fluorescent product from the mixture of cefazolin sodium (2.4 mM), β-lactamase (1.0 U/mL) and **DNBS-CSA** (2.0 mM), which reacted for 30 min in PBS buffer solution (10 mM, pH 7.4, 37 ℃), suggesting the formation of **CSA** from **DNBS-CSA.**

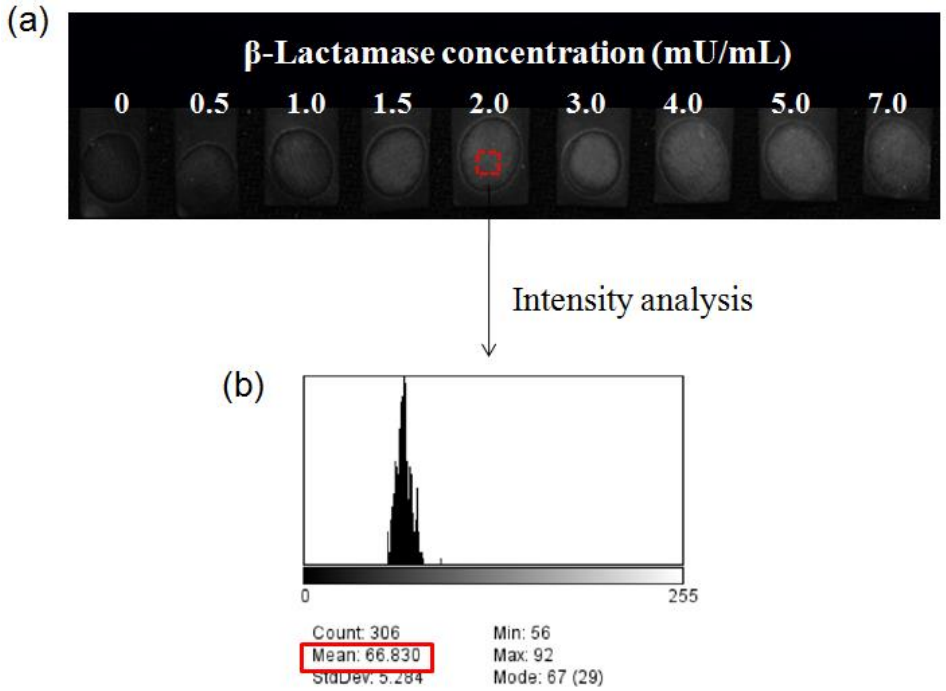

Fig. S4 (a) The corresponding black-and-white photographs of Fig. 4a. (b) Intensity analysis of fluorescent spots by using Image J software.

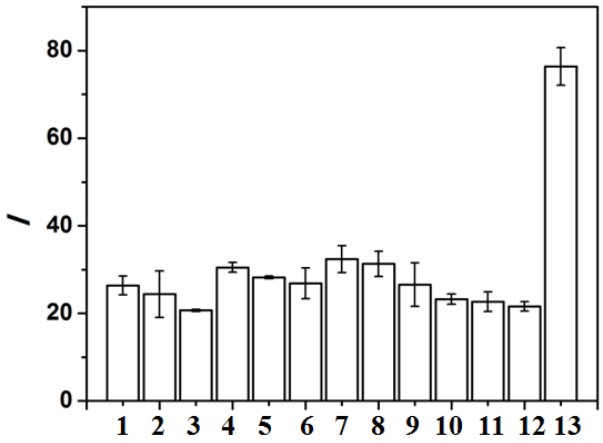

Fig. S5 (a) Fluorescence intensity of **DNBS-CSA** test paper upon addition of various species. 1: blank; 2: $CaCl_2$ (50 μM); 3: Mg $(NO_3)_2$ (50 μM); 4: $Zn(NO_3)_2$ (50 μM); 5: $Fe(NO_3)_3$ (50 μM); 6: $Cu(NO_3)_2$ (50 μM); 7: pepsin (50 mU/mL); 8: lysozyme (50 mU/mL); 9: vitamin C (50 μM); 10: alanine (50 μM); 11: BSA (50 μg/mL); 12: esterase (50 mU/mL) and 13: β-lactamase (50 mU/mL).

Table S1 Comparison of analytical performance of β-lactamase probes in literatures and this paper.

References	Fluorescence detection type	Detection environment	Detection limit	Reaction time
Anal. Chem., 2014, **86**, 6115-6120	Light-up	PBS solution with pH at 7.4	0.02 nM	15 min
Anal. Chem., 2016, **88**, 5605-5609	Light-up	PBS solution with pH at 7.4	1×10^{-5} U/mL	15 min
ChemBioChem, 2017, **18**, 1990 -1994	Ratiometric	PBS solution with pH at 7.4	0.5 pM	30 min
Food Control, 2017, **73**, 726-733	Light-up	Tris-HCl buffer solution with pH at 7.2	0.5 U/mL	20 min
This paper	Light-up	PBS solution with pH at 7.4 or on test papers	5×10^{-4} U/mL	8 min in solution or 20 min on test paper

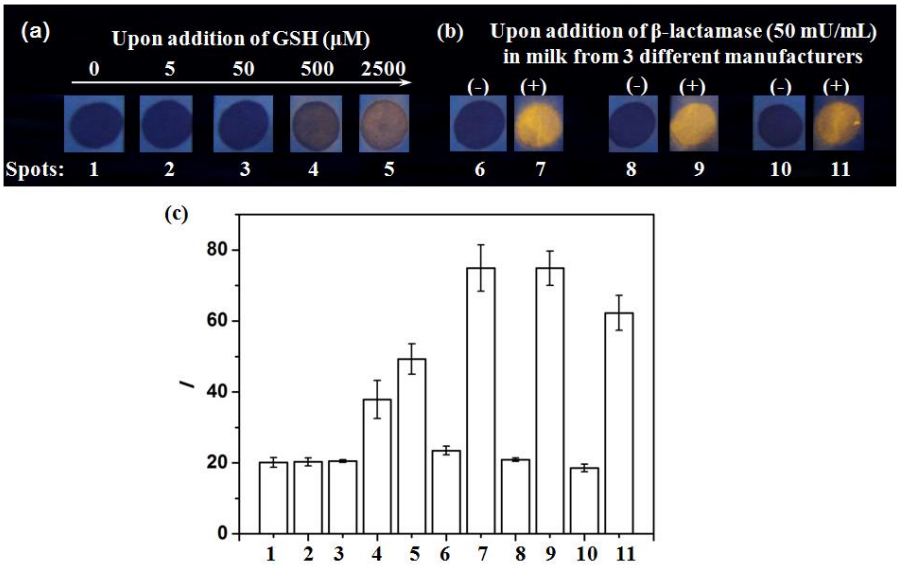

Fig. S6 (a) Photographs of **DNBS-CSA** test paper under a UV lamp (365 nm) upon addition of GSH (0-2500 μM) in 10 mM PBS solution at pH 7.4. (b) Photographs of **DNBS-CSA** test paper for sample solution containing 10% milk from three different manufacturers upon addition of β-lactamase. (-) and (+) means without or with addition of β-lactamase (50 mU/mL) and cefazolin sodium (4.8 mM), respectively. (c) The corresponding fluorescence intensity of spots of (a) and (b) sequentially, read by Image J software. 1-5: The response of **DNBS-CSA** test papers to GSH. 6-11: The response of **DNBS-CSA** test papers to milk without or with addition of β-lactamase. This comparison experiment shows that the concentration of biological thiols in milk should be too low to turn on the fluorescence of **DNBS-CSA.**

Table S2 Selectivity study of the interfering species at the concentration level reported in milk.

Components	Mass concentration in milk (mg/kg)	Molar concentration in milk (mM)	Chosen concentration for selectivity study
Lactose	$4\text{-}5\times10^4$ [2]		50 mg/mL
Casein	2.5×10^4 [3]		25 mg/mL
Mg	105-240 [4]	4.4-10	10 mM
Zn	2.56-6.73 [4]	0.04-0.11	0.1 mM
Fe	5.62-11.02 [5]	0.1-0.2	0.2 mM
Cu	0.57-0.69 [5]	0.009-0.01	0.01 mM

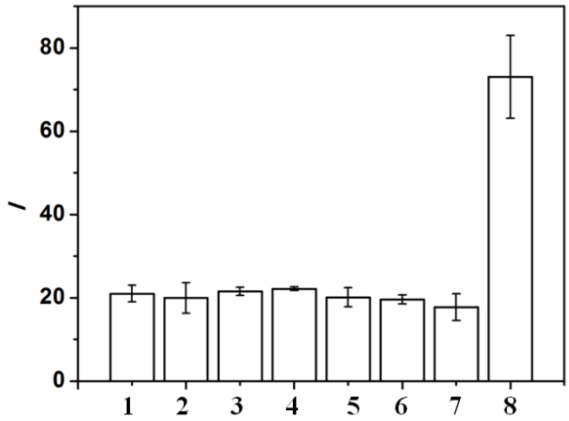

Fig. S7 Fluorescence intensity of **DNBS-CSA** upon addition of common interfering species in milk. 1: blank; 2: lactose (50 mg/mL); 3: casein (25 mg/mL); 4: Mg^{2+} (10 mM); 5: Zn^{2+} (0.1 mM); 6: Fe^{3+} (0.2 mM); 7: Cu^{2+} (0.01 mM); 8: β-lactamase (50 mU/mL).

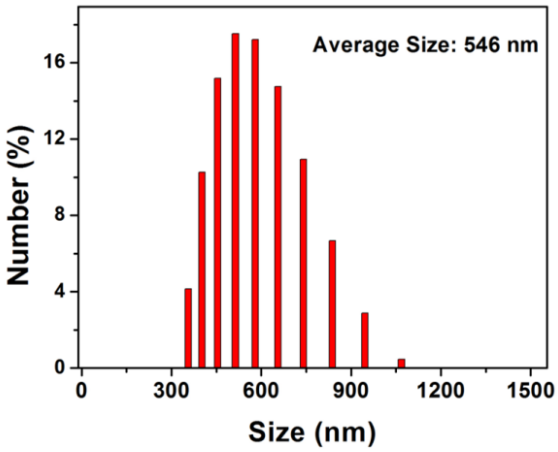

Fig. S8 Dynamic lighter scattering (DLS) analysis of **DNBS-CSA** (150 μM) in PBS buffer solution (10mM, pH 7.4) containing 30% DMSO.

Compound **DNBS-CSA** and **CSA** was prepared through our early reported procedure[1]. The molecular structures were confirmed to be right by the NMR and MS spectroscopy.

For **DNBS-CSA**, ^{1}H NMR (300 MHz, DMSO-d_6): 10.87 (s, 1H), 9.11 (d, 1H, J = 2.4 Hz), 8.85 (s, 1H), 8.60 (m, 2H), 8.33 (d, 1H, J = 8.6 Hz), 8.06 (d, 1H, J = 2.8 Hz), 7.69 (m, 2H), 7.41 (m, 2H), 6.96 (m, 2H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 164.28, 159.30, 155.00, 152.12, 148.65, 146.68, 134.44, 134.26, 133.73, 133.26, 131.01 (2C), 129.44, 128.25, 128.13, 126.18, 121.55, 120.16, 118.59, 117.08. ESI mass spectrometry: calc. for $C_{20}H_{13}ClN_4NaO_8S$ $[M+Na]^+$ 527.0040, found 527.0035.

For **CSA**, ^{1}H NMR (300 MHz, DMSO-d_6): 11.15 (s, 1H), 11.07 (s, 1H), 9.00 (s, 1H), 8.93 (s, 1H), 7.76 (d, 1H, J = 2.4 Hz), 7.69 (dd, 1H, J_1 = 7.56 Hz, J_2=7.53 Hz), 7.42 (t, 1H), 7.39 (dd, 1H, J1 = 6.18 Hz,J_2=5.16 Hz), 6.97 (m, 3H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 163.75, 161.04, 159.23, 157.77, 133.92, 133.11, 131.26, 129.32 123.65, 120.51, 120.15, 119.01, 118.72, 117.02. ESI mass spectrometry: calc. for $C_{14}H_{10}ClN_2O_2$ $[M-H]^-$ 273.0431, found 273.0434.

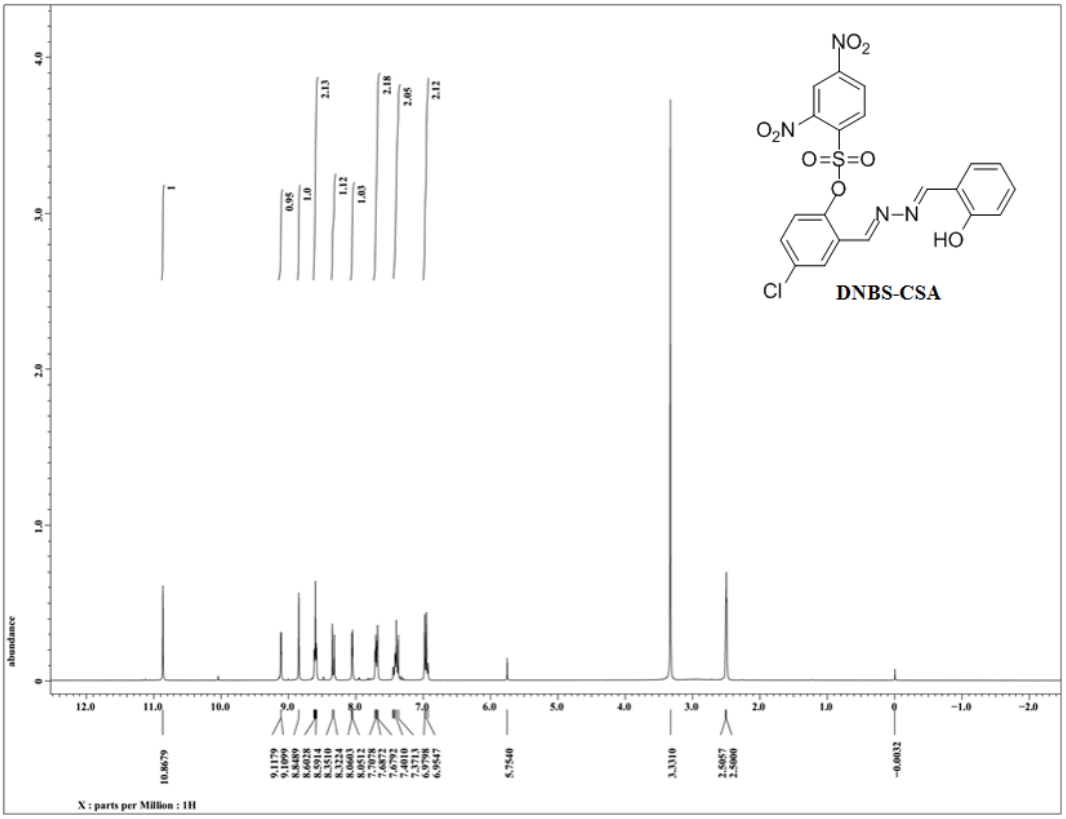

Fig. S9 ^{1}H NMR spectrum of compound **DNBS-CSA**.

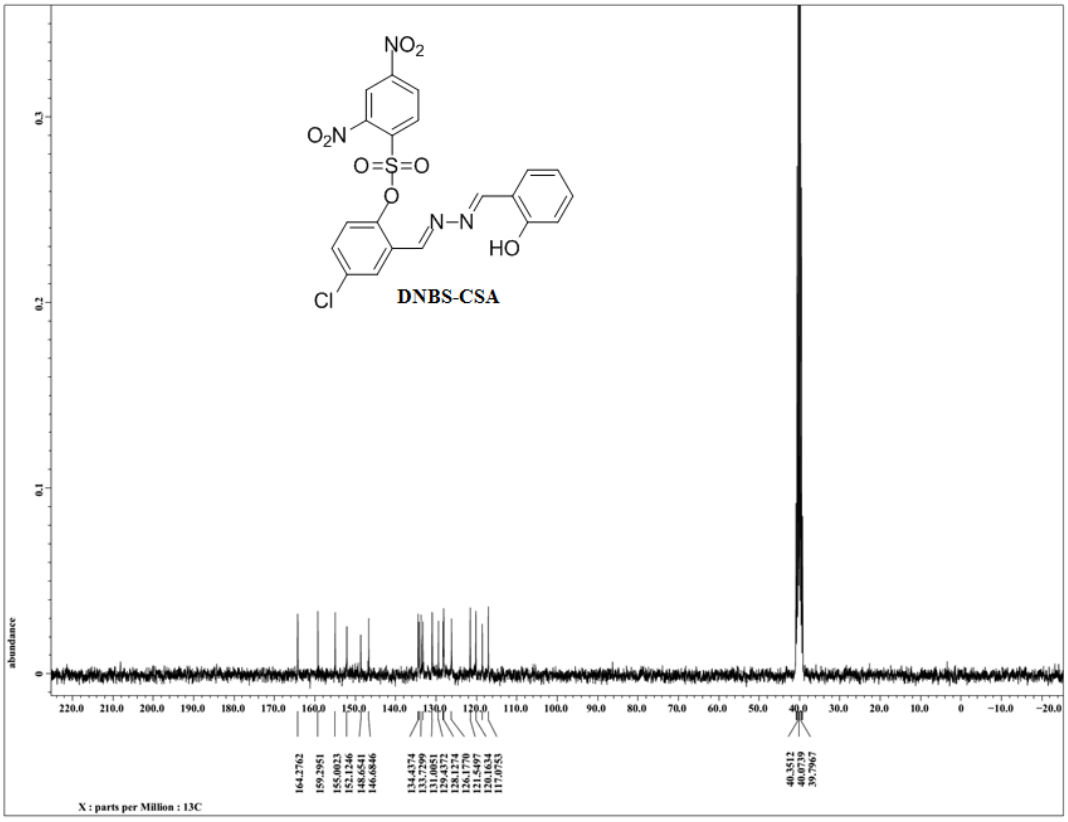

Fig. S10 ^{13}C NMR spectrum of compound **DNBS-CSA**.

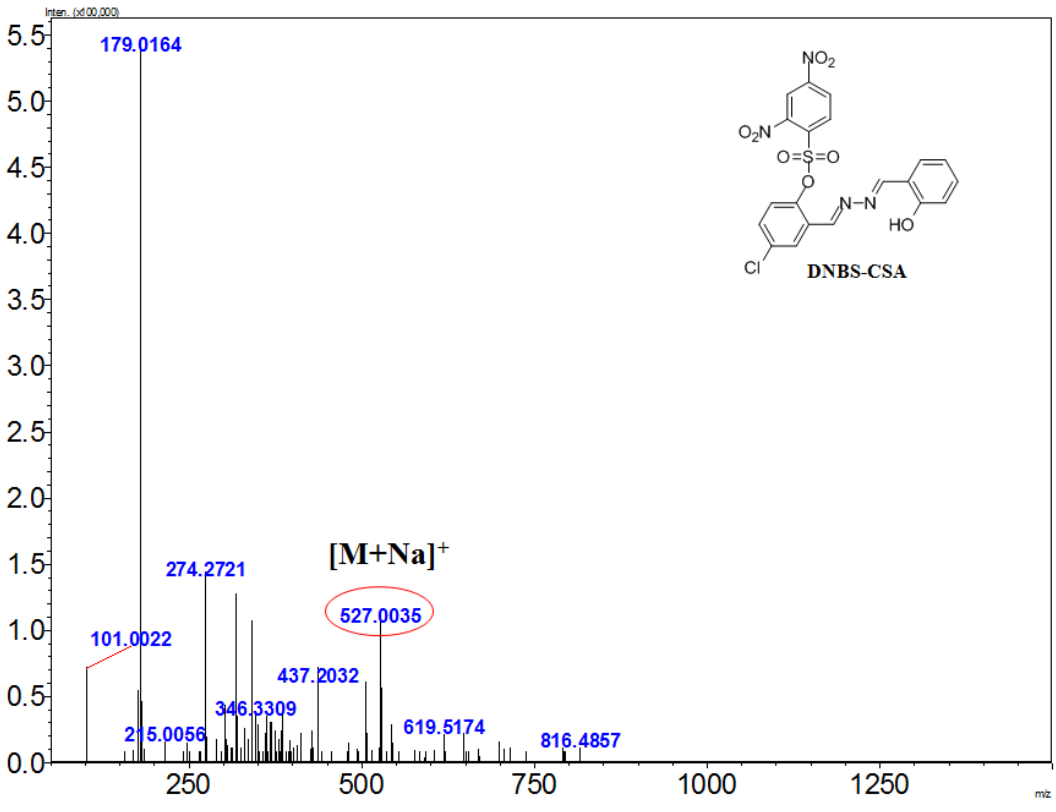

Fig. S11 Mass spectrum of **DNBS-CSA**.

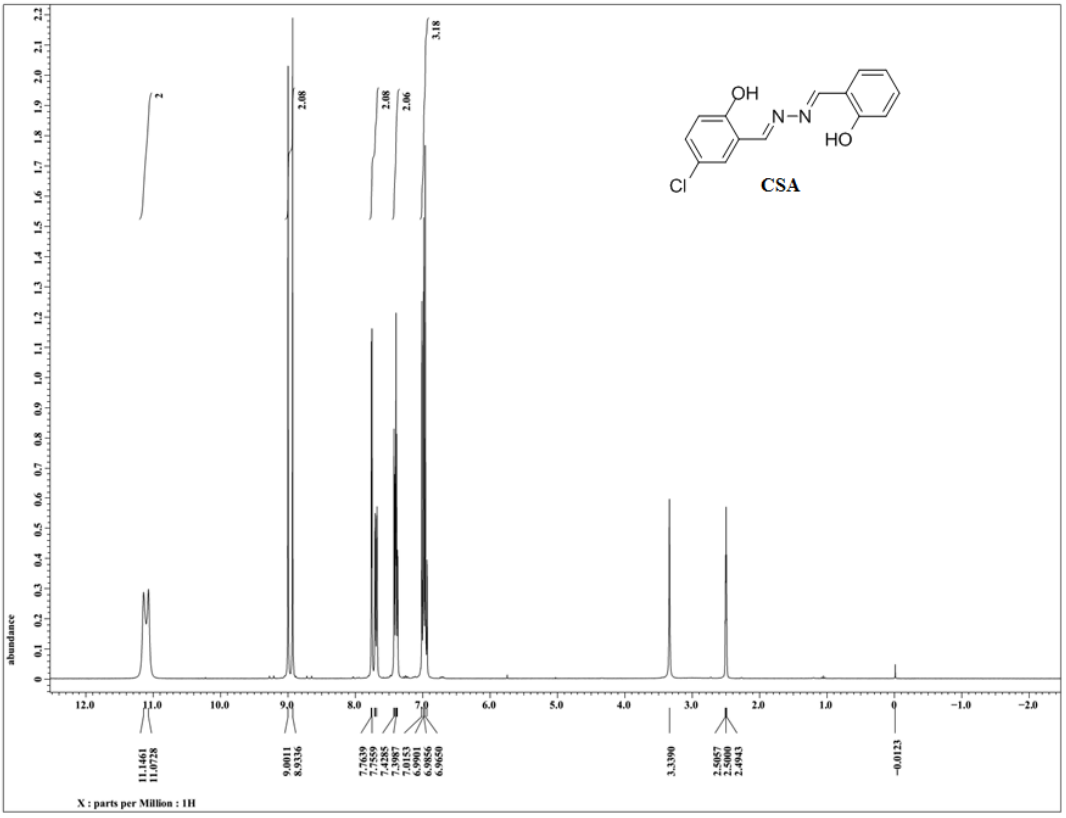

Fig. S12 ^{1}H NMR spectrum of compound **CSA**.

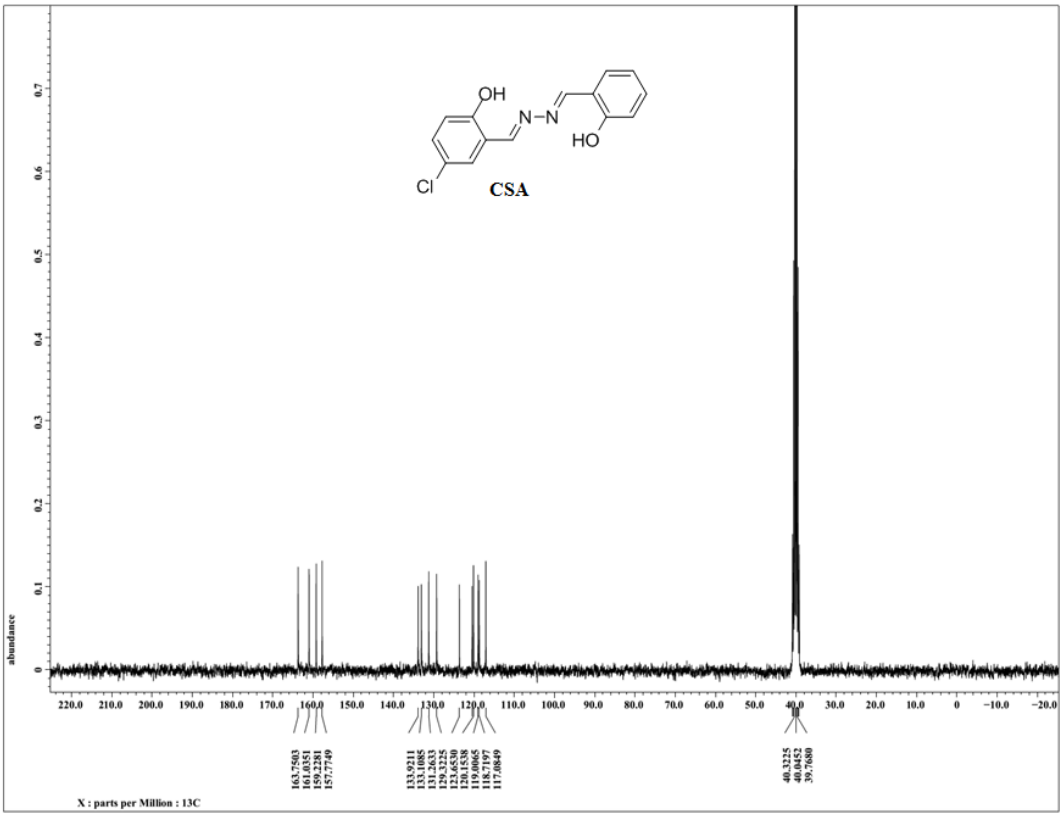

Fig. S13 ^{13}C NMR spectrum of **CSA**.

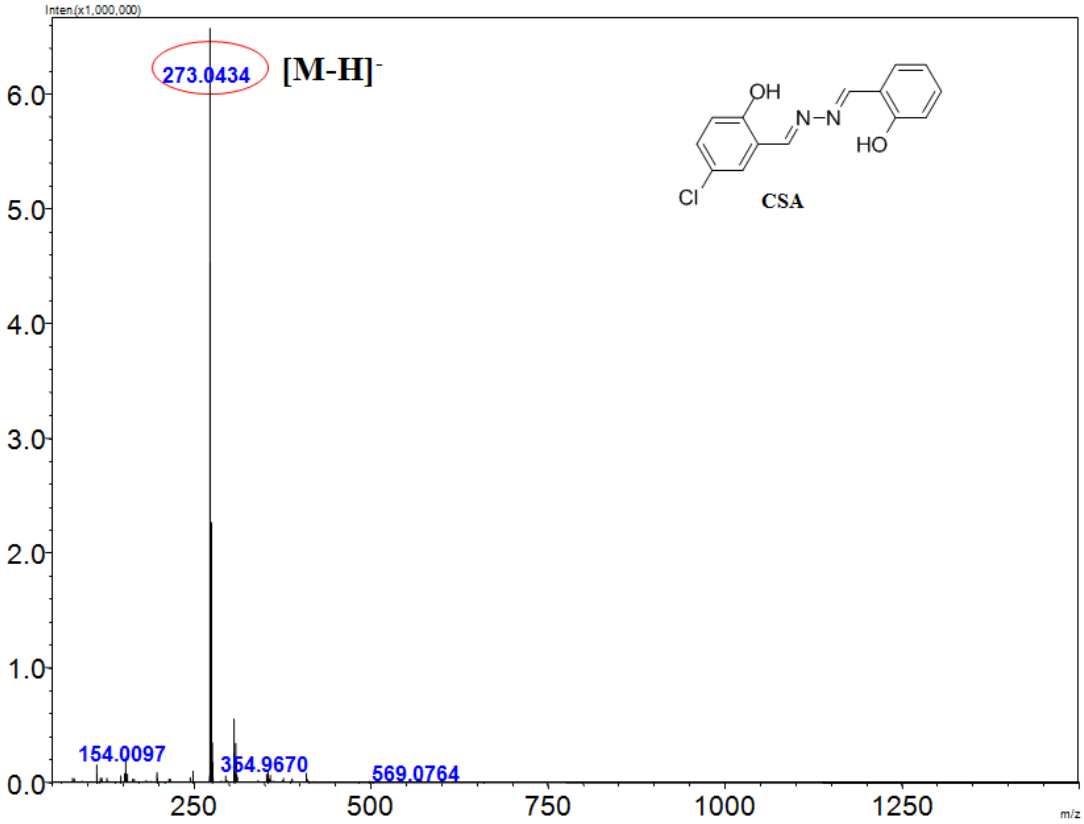

Fig. S14 Mass spectrum of **CSA**.

References

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