Supporting Information for

Piperazine-tuned NBD-based colorimetric and fluorescent turn-off probes for hydrogen sulfide

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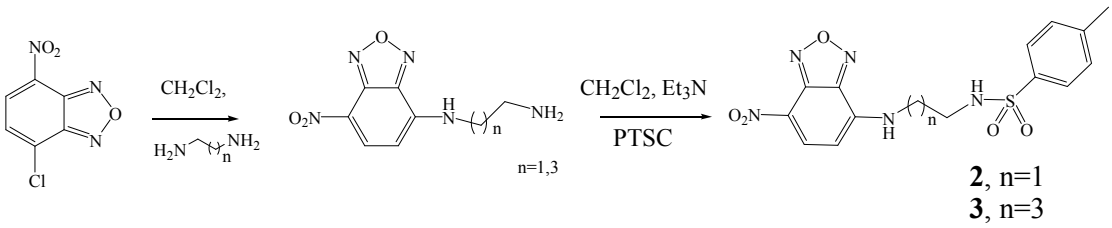
1. Synthesis of other compounds

1.1 *Synthesis of* NBD-Pz

The compound **NBD-Pz** was synthesized according to the literature as dark orange product. A red solid was obtained, yield: 62%; M.p: 134-135 °C; ESI-HRMS (m/z): $[M+Na]^+$ calcd: 272.0778, found: 272.0781; ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.44 (d, J = 9.2 Hz, 1H), 6.64 (d, J = 9.3 Hz, 1H), 4.08 (s, 4H), 2.95-2.93 (m, 4H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 145.75, 145.43, 145.25, 136.72, 120.91, 103.65, 51.73, 46.23.

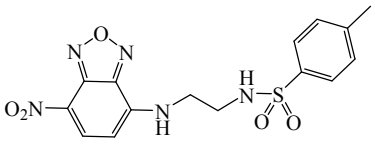
1.2 *Synthesis of* 2 and 3



Scheme S1 Synthesis of probes **2** and **3**

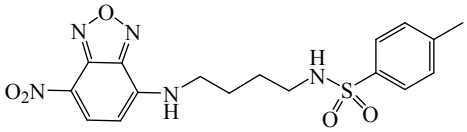
To a round-bottomed flask (50 mL) was added 10 mmol of diamine and 30 mL CH_2Cl_2. Then NBD-Cl (1 mmol) was added. The reaction was stirred at room temperature for 30 min. The resulting solution was evaporated to dryness under reduced pressure. The residue was redissolved in CH_2Cl_2 (50 mL) and washed with saturated salt water (3×30 mL). Then $MgSO_4$ was added to remove water and the filtrate was evaporated. The residue was not purified and put into the next step.

The intermediate was dissolved in CH_2Cl_2 (50 mL), and TEA (2 mmol) was added. The reaction mixture was stirred at 0 °C. Then PTSC (1.5 mmol) was added and stirred for 1 hour at 0 °C. The reaction mixture was stirred other 5 hours at room temperature. The resulting solution was evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel with ethyl acetate-petroleum ether to afford the product.



2

M.p: 164-165 °C. ESI-HRMS calcd for $C_{17}H_{17}N_5O_5SNa$ ($[M+Na]^+$): 400.0686; found: 400.0698. ^1H NMR (400 MHz, DMSO) δ 9.22 (s, 1H), 8.47 (d, *J* = 8.8 Hz, 1H), 7.75 (s, 1H), 7.62 (d, *J* = 7.6 Hz, 2H), 7.24 (d, J = 7.4 Hz, 2H), 6.32 (d, *J* = 8.5 Hz, 1H), 3.54 (s, 2H), 3.11 (q, *J* = 5.5 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 145.48, 144.93, 144.45, 143.24, 138.28, 137.87, 129.94, 126.93, 121.49, 99.62, 43.38, 41.24, 21.28.



3

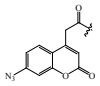
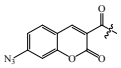
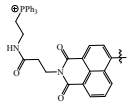
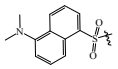
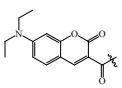
M.p: 189-190 °C.ESI-HRMS calcd for $C_{17}H_{17}N_5O_5SNa$ ($[M+Na]^+$): 428.0999; found: 428.1007. ^1H NMR (400 MHz, DMSO) δ 9.51 (s, 1H), 8.49 (d, *J* = 8.6 Hz, 1H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.52 (t, *J* = 5.5 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 2H), 6.38 (d, *J* = 8.8 Hz, 1H), 3.42 (d, *J* = 4.6 Hz, 2H), 2.76 (q, *J* = 6.3 Hz, 2H), 2.35 (s, 3H), 1.64 (m, 2H), 1.48 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 145.69, 144.89, 144.61, 142.96, 138.38, 138.10, 130.04, 126.93, 121.05, 99.61, 43.28, 42.60, 26.89, 25.25, 21.38.

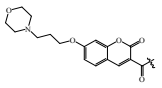
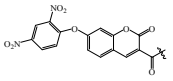
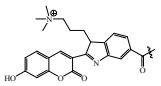
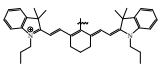
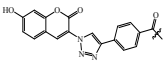
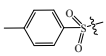
2. General procedure of MTT assay

The MTT assay was used to test the cytotoxicity of 1 to HeLa cells. Hela cells were cultured in a 96-well plate. Various concentrations of **1** were added to the wells and the cells were incubated at 37 °C under an atmosphere of 5% CO_2 for 48 h. 10 μM 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was added to each well and incubated at 37 °C under 5% CO_2 for 4 h. Remove the MTT solution and yellow precipitates (formazan) observed in plates were dissolved in 100 mL DMSO. The absorbance of 570 nm was measured for each well using microplate reader. The viability of cells was calculated according to the following equation: Cell viability = A570 (sample) / A570 (control).

3. Supporting figures

Table S1 Fluorescent probes of piperazinyl-NBD derivatives for detecting H_2S.

Compound	R	$\kappa_{obs}(s^{-1})([H_2S])$	$\kappa_2(M^{-1}s^{-1})$	Ref.
NBD-Pz	**H**	4.4×10^{-3}(75μM)	43.26	Org. Biomol. Chem. **2016**, 14, 11117.
		2.0×10^{-3}(200μM)	21.77	
A1		ND	ND	Chem. Asian J. **2016**, 11, 1376
A2		ND	ND	
A4		2.04×10^{-3}(100μM)	ND	Anal. Chem. **2016**, 88, 5476
A5		ND	ND	Org. Biomol. Chem. **2015**, 13, 9760
A6		1.1×10^{-3}(500μM)	2.2	Tetrahedron Lett. **2013**, 54, 6937.

A7		ND	25.0(pH=7.4);6.8(pH=6.8)	Tetrahedron **2015**, 71, 8572
A9		ND	ND	Chem. Commun. **2015**, 51, 7505
A10		ND	ND	Tetrahedron Lett. **2015**, 56(8):1015.
A11		ND	14.9	*Chem. Sci.* **2017**, *8*, 2776
A12		3.3×10^{-3}(100μM)	33(Na_2S);37(NaHS)	Tetrahedron Lett. **2016**, 57, 1187
1		2.53×10^{-3}(500μM)	0.49	This work

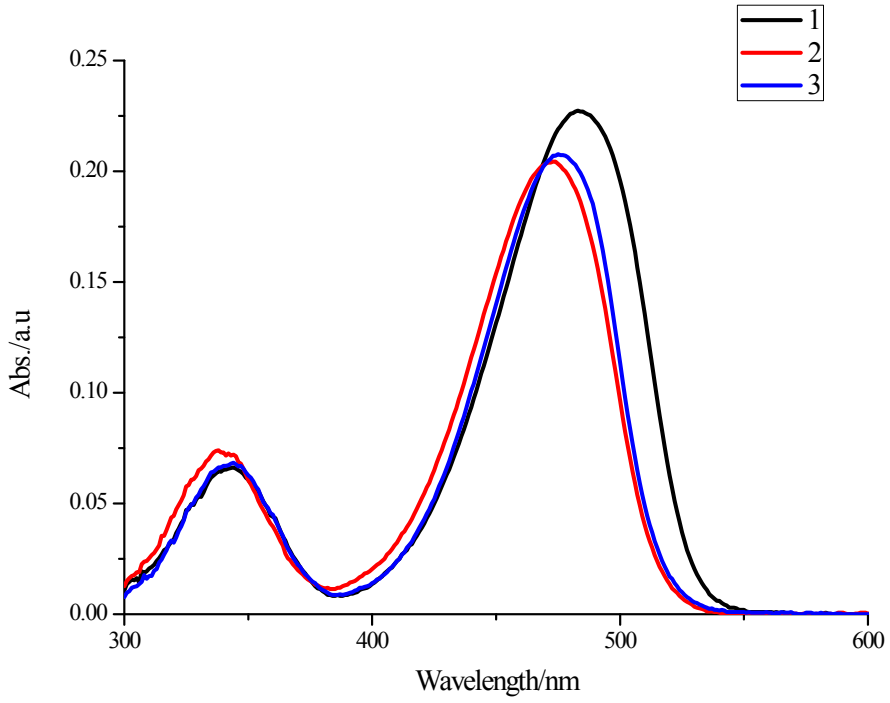


Figure S1. The UV-Vis spectra of **1**-**3** (10 μM) in CH_3CN-PBS (v/v = 1:2, pH=7.4, 10 mM) solution at room temperature.

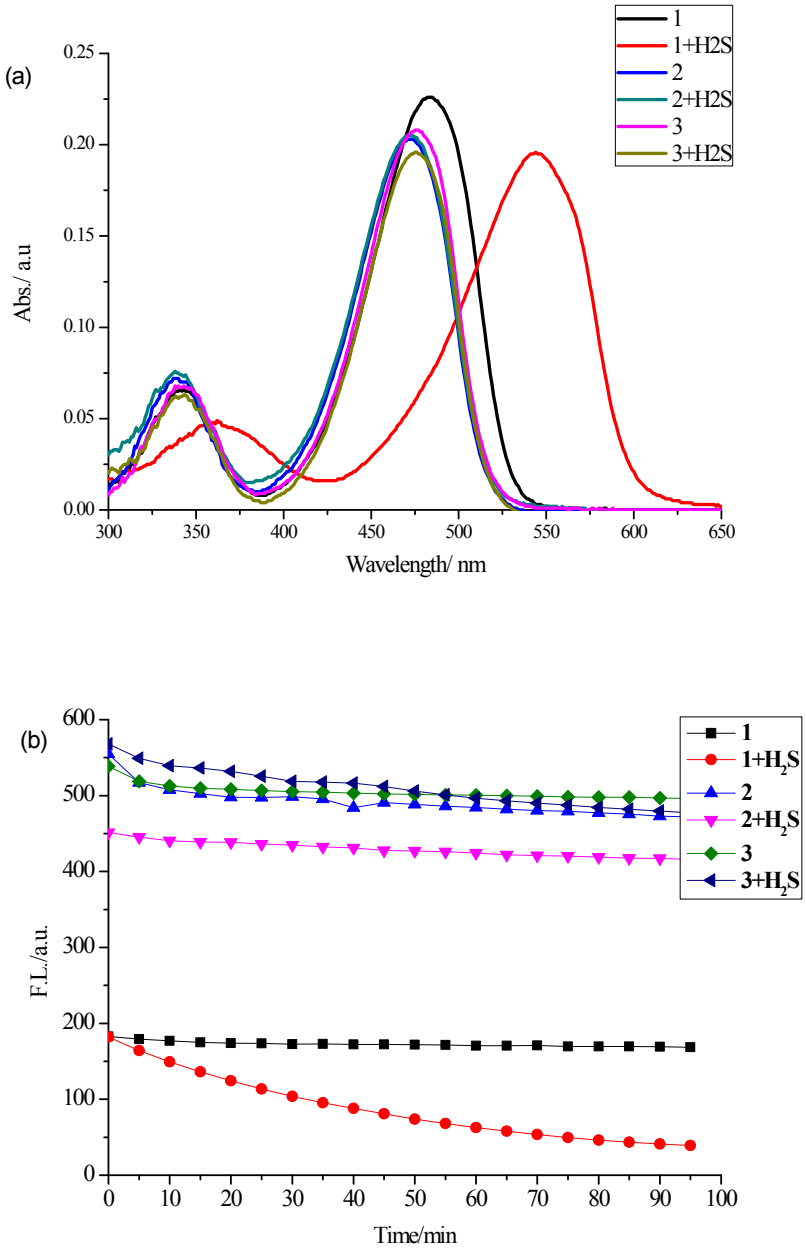


Fig S2. (a) The UV-Vis spectra of **1-3** (10 μM) and UV-Vis spectral change of **1-3** with H_2S in CH_3CN-PBS (v/v = 1:2, pH=7.4, 10 mM) solution at room temperature. (b) The fluorescent spectra of **1-3** against time and the fluorescent spectral change of **1-3** with H_2S against time in CH_3CN-PBS (v/v = 1:2, pH=7.4, 10 mM) solution at room temperature.

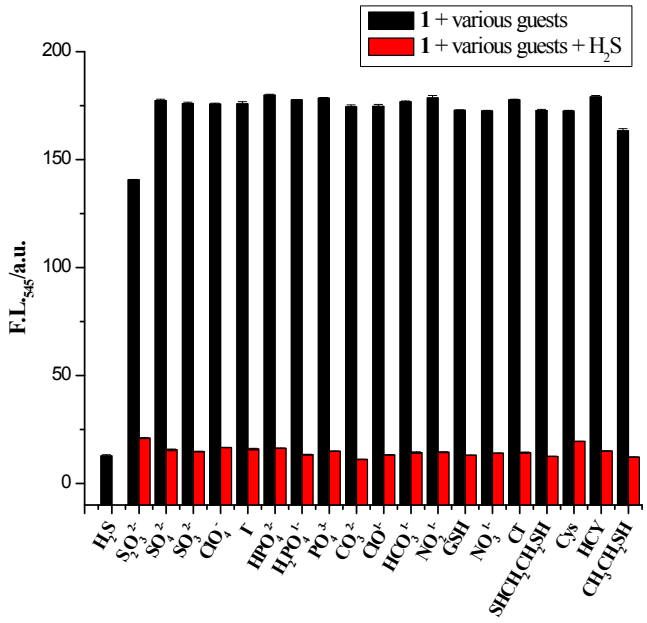
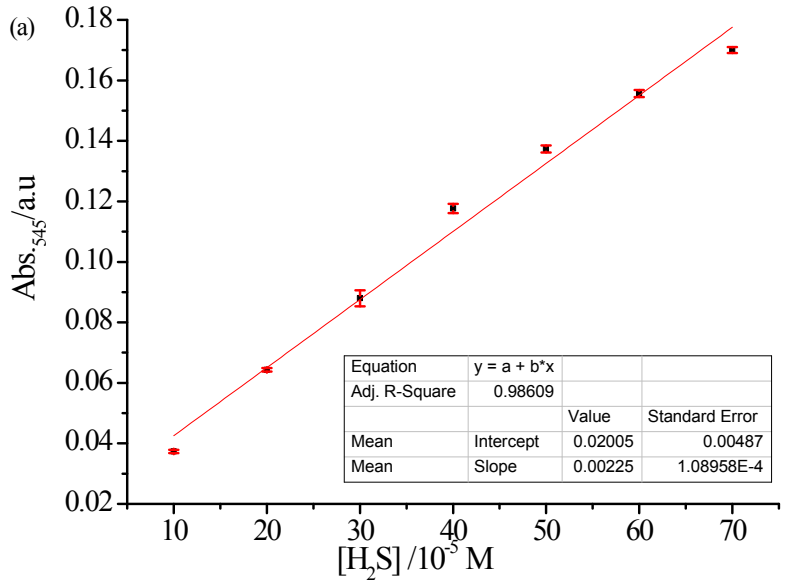


Fig S3. Fluorescence response of **1** (10 μM) with various guests. Black bar: **1** + various guests. Red bar: **1** + various guests + H_2S. (λ_{ex}=485 nm, λ_{em} =545 nm, slit: 10/10 nm).



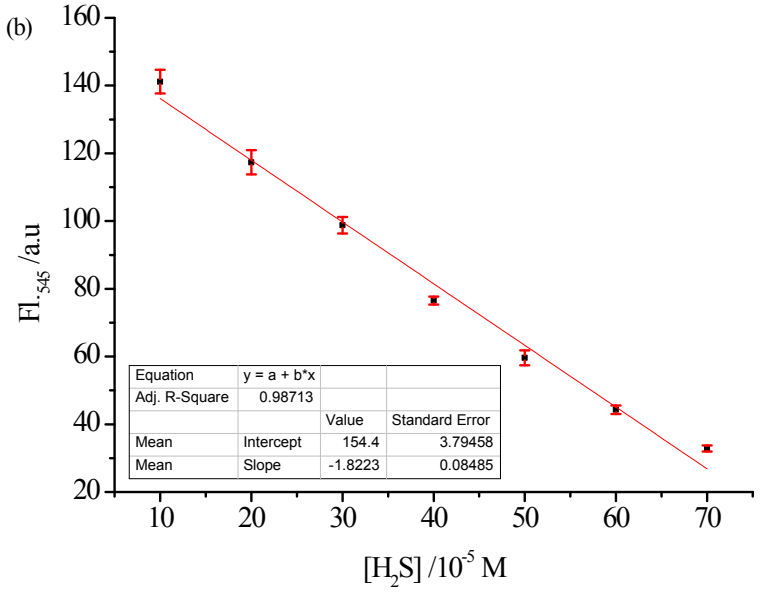


Fig S4. (a) The UV-Vis absorption spectra of **1** (10 μM) at 545 nm upon the titration of H_2S (100, 200,300,400, 500, 600, 700 μM) in CH_3CN-PBS (v/v = 1:2, pH=7.4, 10 mM) solution at room temperature. (b) The fluorescence emission spectra of **1** (10 μM) with the emission at 545 nm upon the titration of H_2S (100, 200,300,400, 500, 600, 700 μM) in CH_3CN-PBS (v/v = 1:2, pH=7.4, 10 mM) solution at room temperature (λ_{ex}=485 nm).

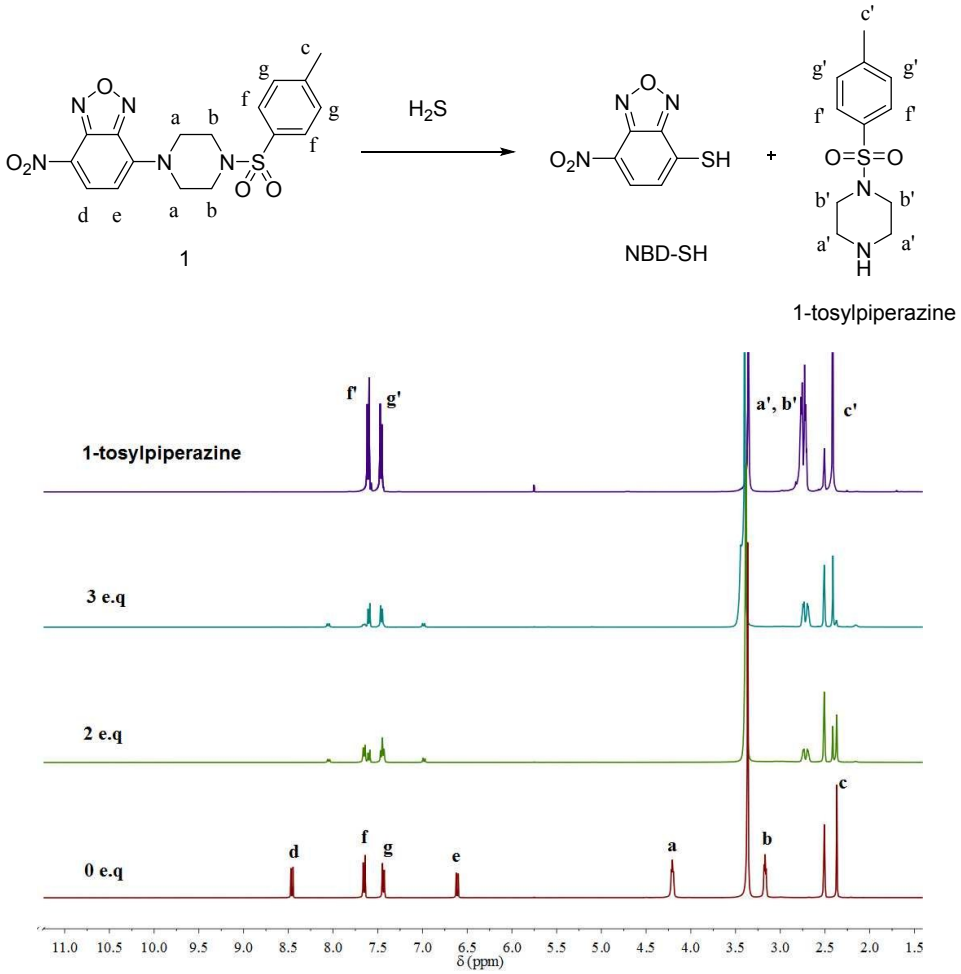


Fig S5. ^{1}H-NMR spectra of **1** with H_2S in DMSO-*d*6 (containing 1% D_2O).

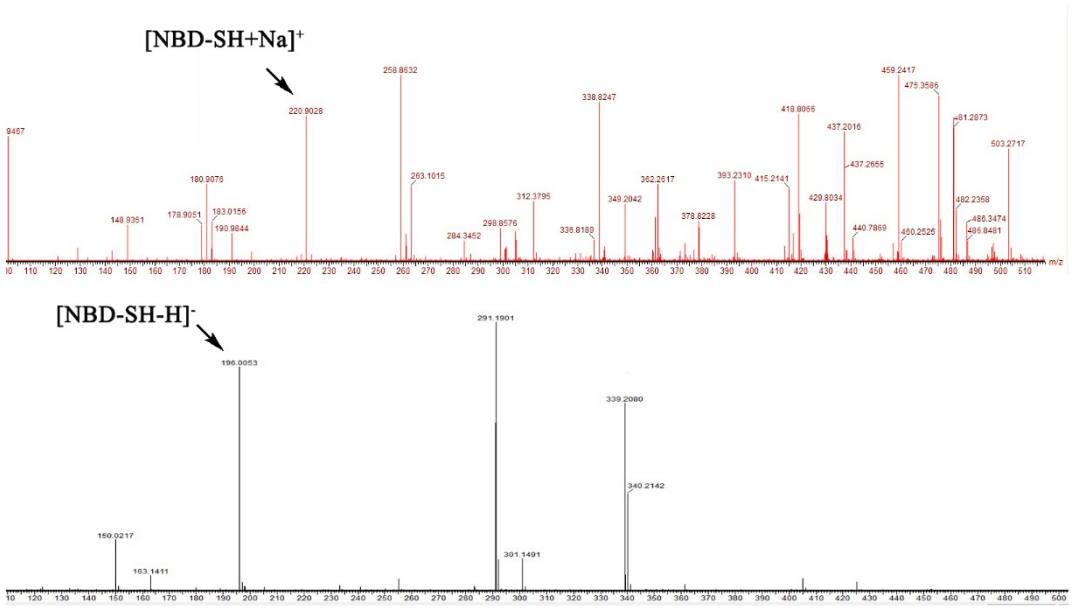


Fig S6. ESI-HRMS of the reaction solution of **1** in the present of H_2S.

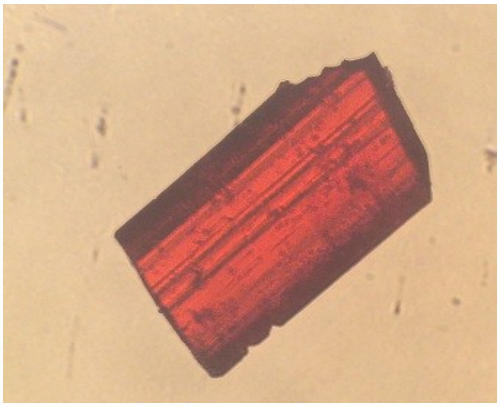


Fig S7. Picture of red crystal of **1**.

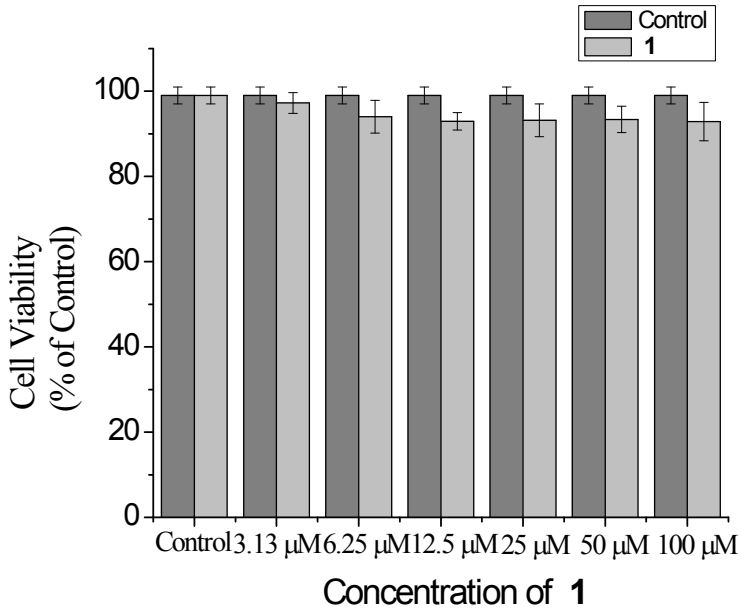
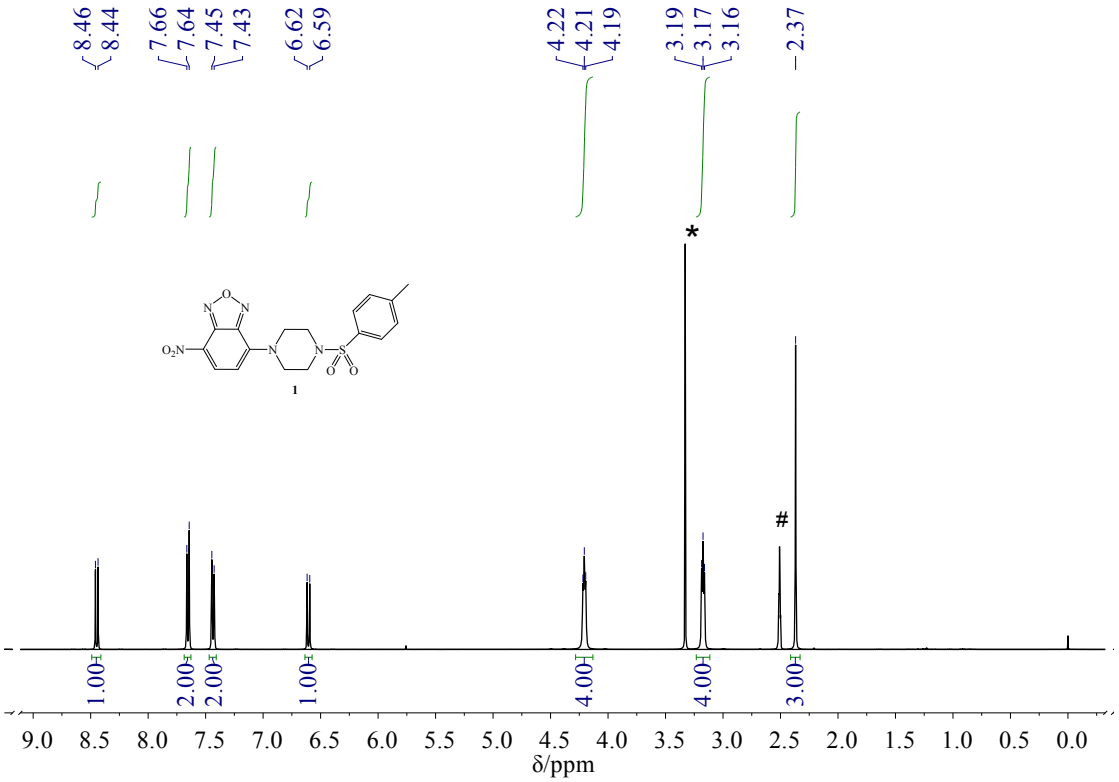
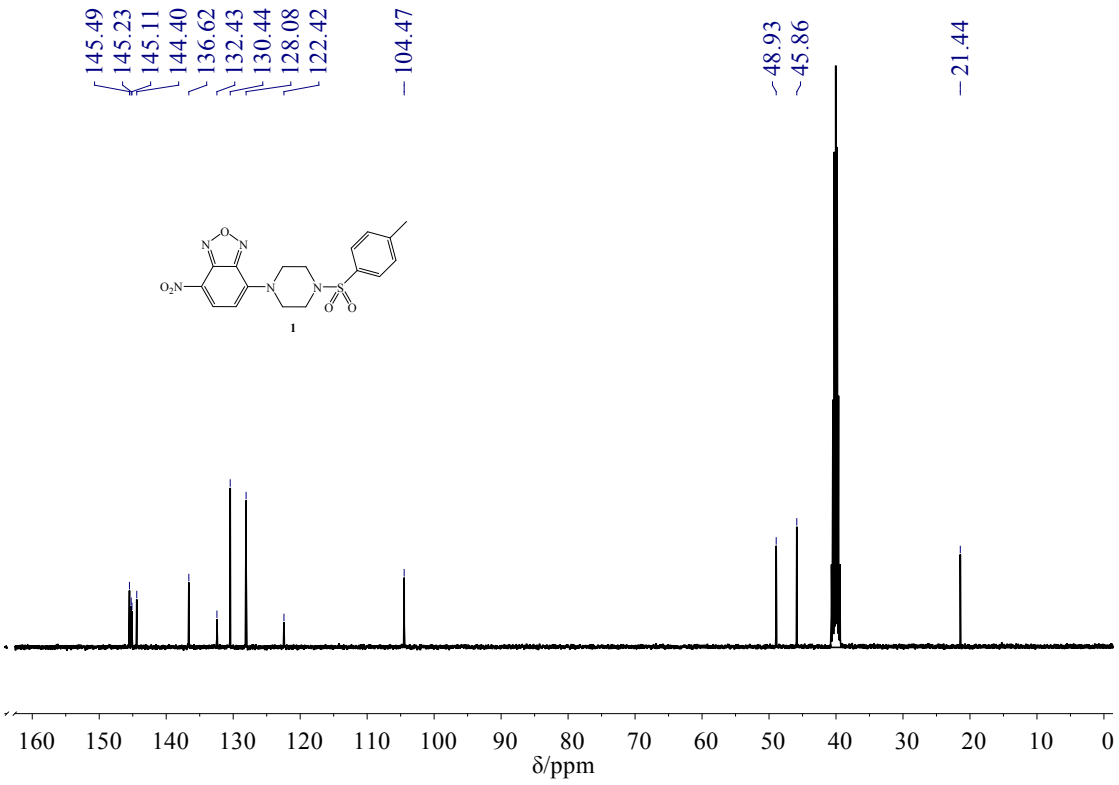
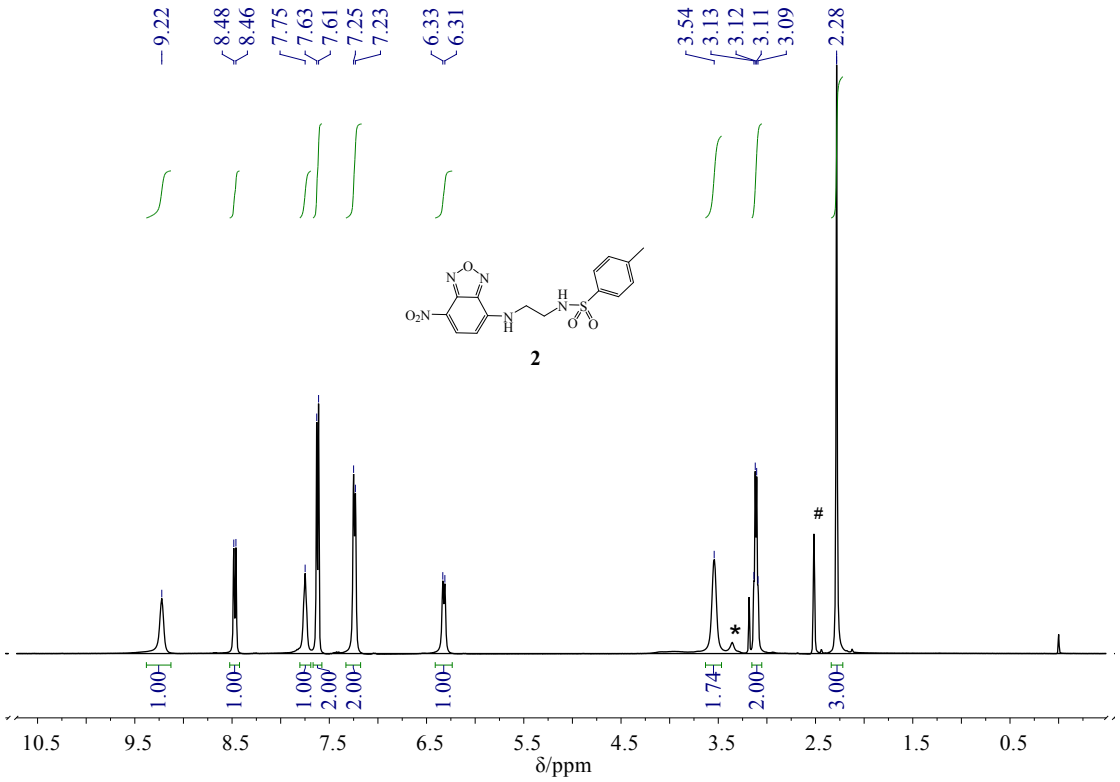


Fig S8. Cytotoxicity of 1 in Hela cells. Cells were treated with different concentrations of 1 for 24 h and cell viability assay were determined by MTT assay. Data were expressed as means ± SD.

Supporting NMR and MS spectra



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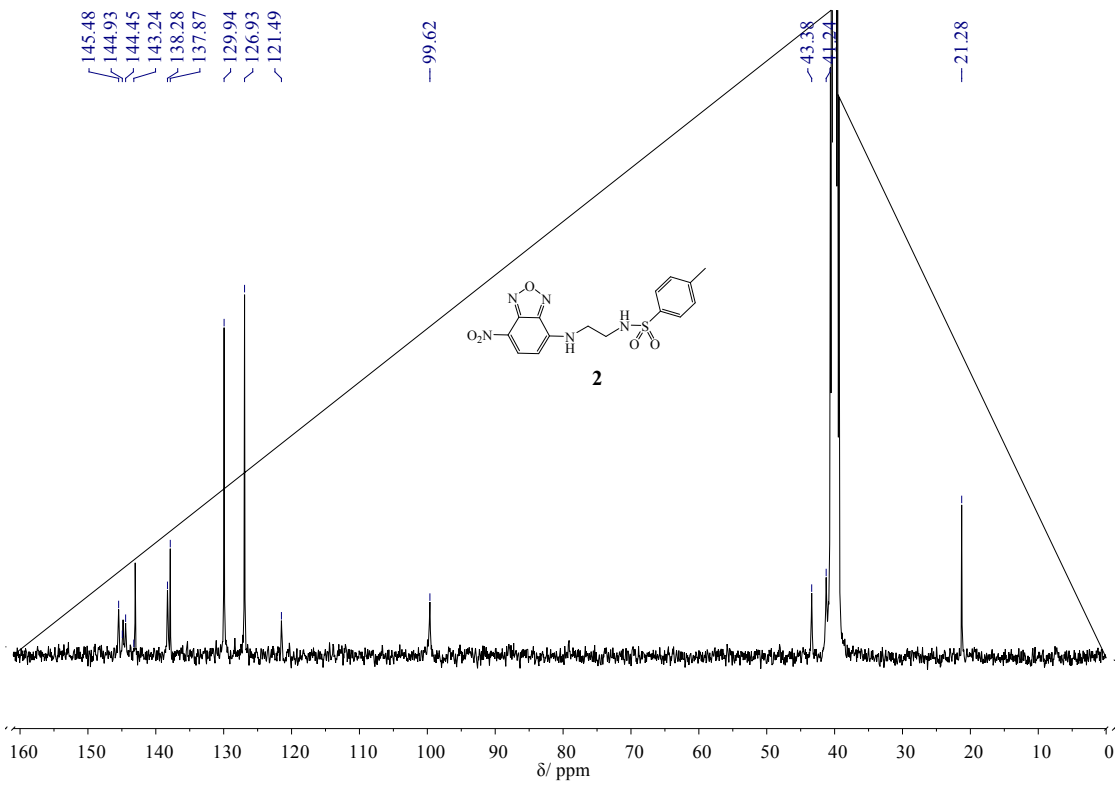
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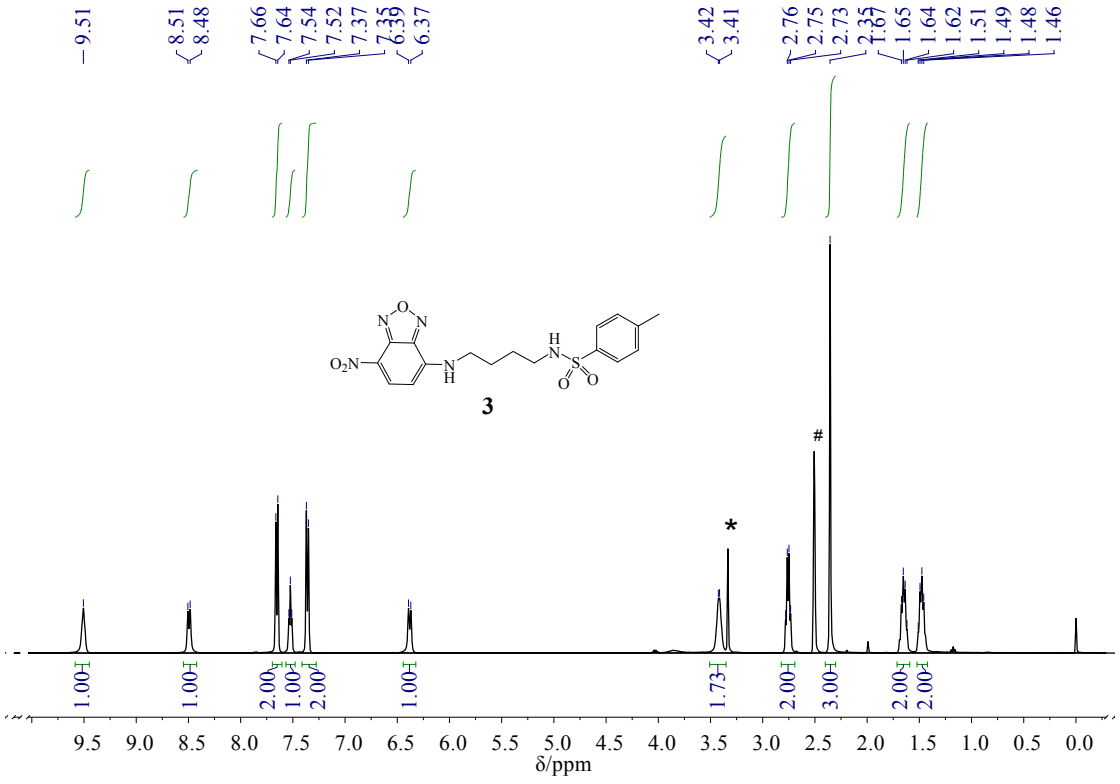
δ/ ppm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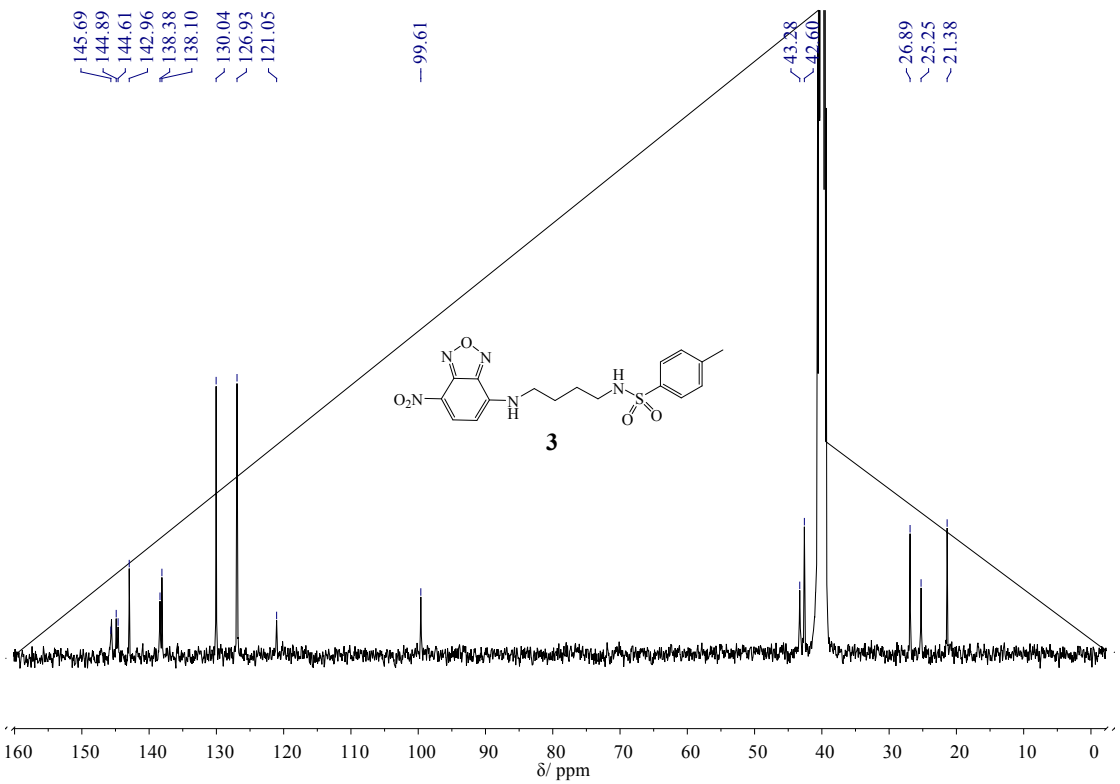
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21.38
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
δ/ ppm

3