## Supplementary data

## A Novel Nopinone-based Turn-on Fluorescent Probe for Hydrazine in Living Cells with High Selectivity

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## **Figure Captions.**

Fig. S1. HRMS of compound 2, compound 3 and probe 4.

Fig. S2. <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 2.

Fig. S3. <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 3.

Fig. S4. <sup>1</sup>H and <sup>13</sup>C NMR spectra of probe 4.

Fig. S5. MTT assay of Hela cells were incubated with 60, 80, 100, 120 and 150  $\mu$ M probe 4 for 24 h.

Fig. S6. Time-dependent fluorescence changes of probe 4 (100  $\mu$ M) upon addition of N<sub>2</sub>H<sub>4</sub> (900  $\mu$ M). ( $\lambda_{ex}$ = 300 nm,  $\lambda_{em}$ = 442nm).

Fig. S7. Absorption changes of Probe 4 (100  $\mu$ M) with N<sub>2</sub>H<sub>4</sub> (0-600 $\mu$ M). All data were measured after 30 min of pretreatment of Probe 4 with N<sub>2</sub>H<sub>4</sub> in ethanol/H<sub>2</sub>O (1/1, v/v) (10 mM phosphate buffer solution, pH = 7.4).

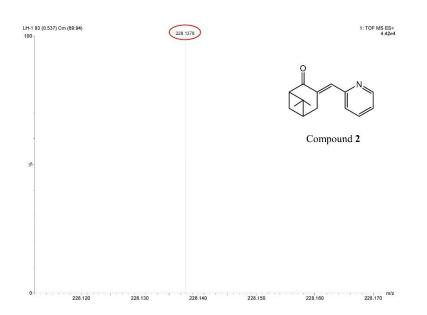
Fig. S8. Fluorescence emission spectra of Probe 4 (100  $\mu$ M) before (black line) and after (red line) the addition of N<sub>2</sub>H<sub>4</sub> (400  $\mu$ M). ( $\lambda_{ex}$ = 300 nm,  $\lambda_{em}$ = 442nm).

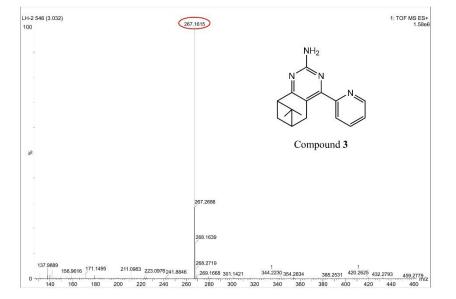
Fig. S9. Absorption spectra of Probe 4 (100  $\mu$ M) in the absence and presence of 600  $\mu$ M hydrazine and Compound 3 in ethanol/H<sub>2</sub>O (1/1, v/v) (10 mM phosphate buffer solution, pH = 7.4).

**Fig. S10.** MTT assay of Hela cells were incubated with 0.2, 0.4, 0.6 and 0.8 M hydrazine for 12 and 24 h.

Fig. S11. Fluorescence spectra of 10, 20, 40, 60, 80 and  $100\mu$ M Probe 4 before (black bar) and after (red bar) the addition of 9 equiv. N<sub>2</sub>H<sub>4</sub>.

Table S1. Comparison of probe 4 with other hydrazine probes.





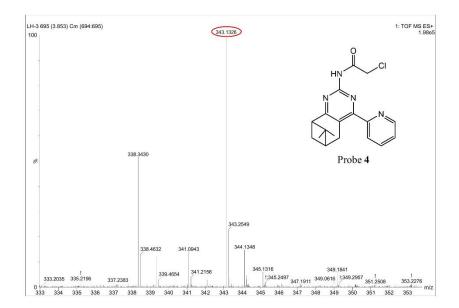
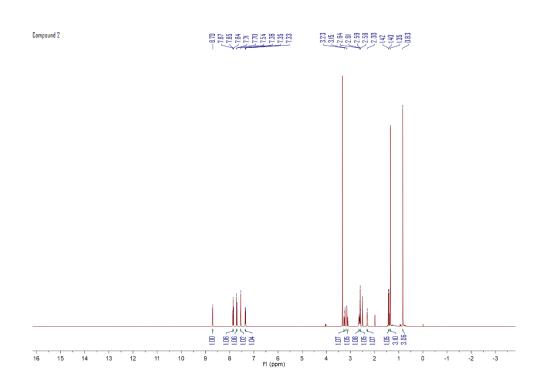
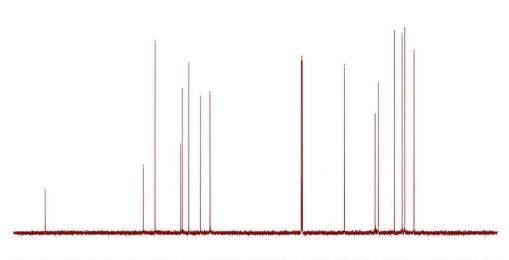


Fig. S1





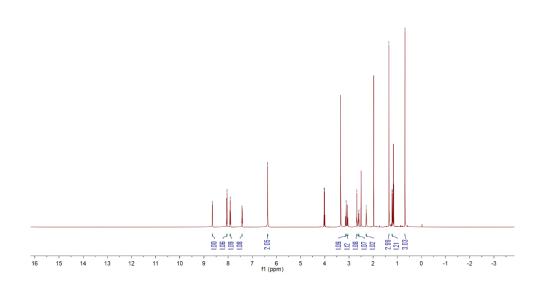


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 f1 (pps)



Compound 3





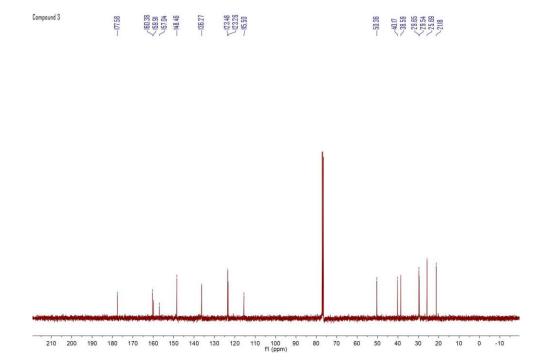
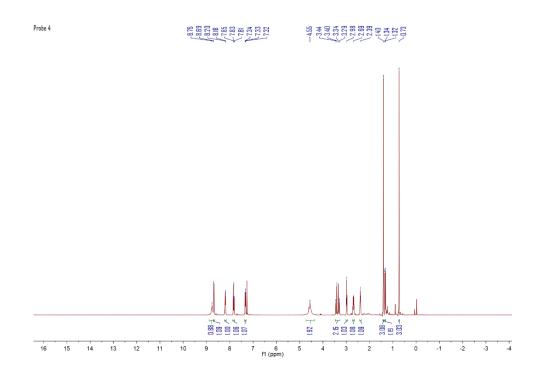


Fig. S3



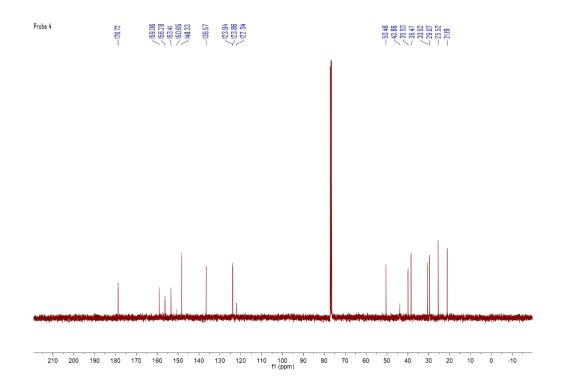


Fig. S4

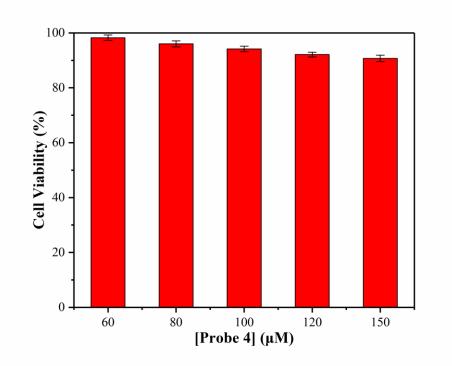


Fig. S5

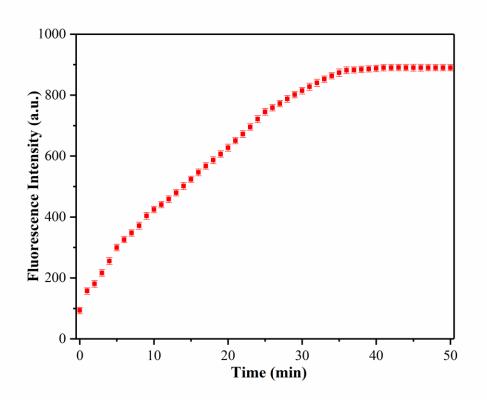


Fig. S6

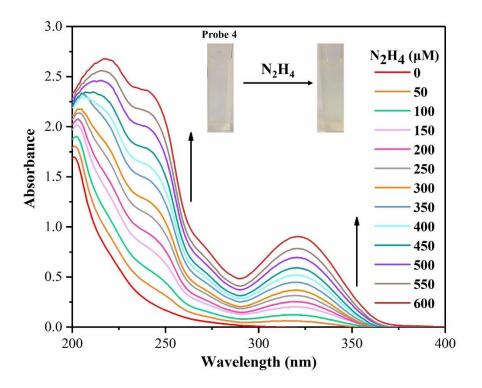


Fig. S7

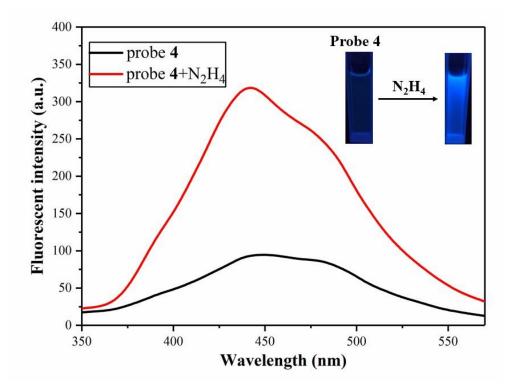


Fig. S8

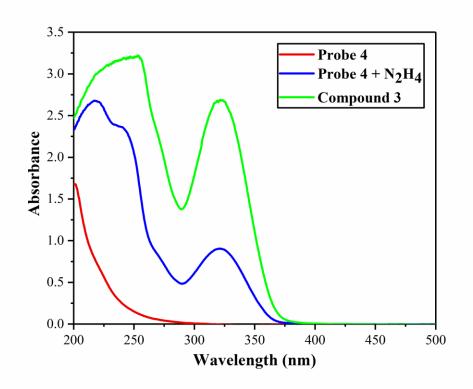


Fig. S9

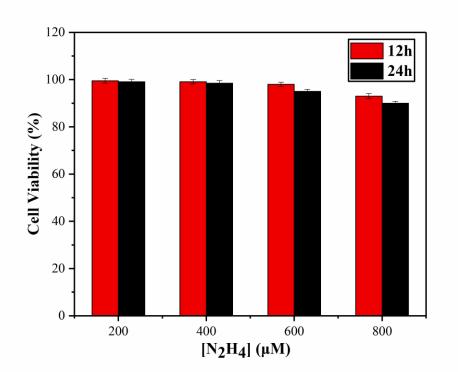


Fig. S10

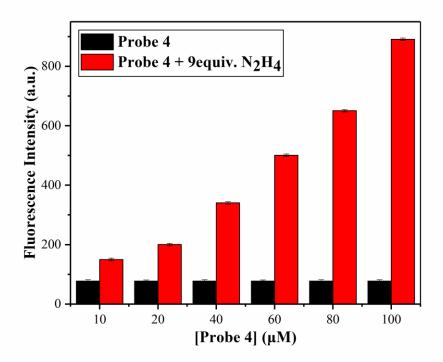


Fig. S11

Structures of Probes for N <sub>2</sub> H <sub>4</sub>	Detection	Solvent	Amounts	Limit of	Cell	Reference No.
	Mode	Medium	of	Detection	Experiment	
			Analytes			
	Turn on	EtOH/HEPES	9	2.9 μM	No	Z. Xu et al.
	Turn on		9	2.9 μM	110	
		(1/1)				Luminescence,
Br						2017, 32(3):
						466-470.
	Ratiometric	EtOH/PBS	27	5.8 nM	Yes	Q. Wu et al.
		(1/5)				Talanta 195
						(2019) 857–864
	Turn on	Acetate	16	Not	No	Myung Gil
		buffer (pH		offered		Choi et al, Org.
		4.5, 10 mM)				Lett., Vol. 13,
		/DMSO (3:7)				No. 19, (2011)
						5260-5263
	Ratiometric	DMSO/H <sub>2</sub> O	12	0.1 μΜ	Yes	Lei Cui et al,
		(7/3)				Anal. Chem.
						2014, 86,
						4611-4617
	Turn on	DMSO/PBS	27	0.46 nM	Yes	Gongchun Li
		(8/2)				et al, J
						Fluoresc.
						(2017) 27:
						323-329
	Ratiometric	DMSO/PBS	24	0.96 µM	Yes	Chuang Liu et
		(2/8)				al,
						Spectrochimica
						Acta
						AVIA

 Table S1. Comparison of probe 4 with other hydrazine probes.

						Part A:
						Molecular and
						Biomolecular
						Spectroscopy.
						212 (2019)
						42-47
	Turn on	EtOH/PBS	54	1.03 μM	Yes	our work
N N N		(1/1)				