Electronic Supplementary Material (ESI) for Dalton Transactions

This journal is © The Royal Society of Chemistry 2019

Supplementary Information For:

A phthalimide functionalized UiO-66 metal-organic framework for the fluorogenic detection of hydrazine in live-cells

Mostakim SK,[a] Mohammed Rafi Uz Zama Khan,[b] Aniruddha Das,[a] Soutick Nandi,[a] Vishal Trivedi[b] and Shyam Biswas[*a]*

[a] Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati, 781039 Assam, India

[b] Malaria Research Group, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, 781039 Assam, India

* To whom correspondence should be addressed. E-mail: sbiswas@iitg.ernet.in; Tel: 91-3612583309.

Experimental Details

Materials and General Methods. The organic linker H_2**L** (2-(1,3-dioxoisoindolin-2-yl)benzene-1,4-dioic acid) was synthesized according to the reported procedure.[1] The other reagents were purchased from various commercial chemical suppliers. X-ray powder diffraction (XRPD) measurements were performed with a Bruker D2 Phaser X-ray diffractometer (30 kV, 10 mA) utilizing Cu-*K*α (λ = 1.5406 Å) radiation. Thermogravimetric (TG) measurements were performed with a SDT Q600 V20.9 Build 20 thermogravimetric instrument within the range of 25-650 °C under N_2 atmosphere at a constant heating rate of 5 °C min^{-1}. The surface morphology of material **1′** was analyzed *via* field-emission scanning electron microscopy (FE-SEM) experiments using a Zeiss Supra 55VP SEM-EDX (SEM = scanning electron microscope) equipment. For checking the optical activity of both H_2**L** and H_2BDC-NH_2 ligands, UV-Vis spectral measurements were conducted with a Perkin Elmer NIR-UV equipment. In order to collect the mass spectrum of the digested material **1′** after treatment with target analytes, an Agilent 6520 Q-TOF high-resolution mass spectrometer (HR-MS) was used. The mass spectrum of **1′** (20 mg) after treatment with hydrazine was recorded after digestion by adding 48% HF (60 μL) to methanol (1.0 mL). A Bruker AM 600 spectrometer was utilized for recording the ^{1}H NMR spectrum of **1′** (20 mg) after digestion in 48% HF/DMSO-d_6 (50 μL/500 μL). For carrying out the N_2 adsorption experiments at −196 °C, a Quantachrome Autosorb iQ-MP volumetric gas adsorption analyzer was employed. The sample was degassed at 120 °C under high vacuum for 24 h before performing the gas adsorption experiments. For investigating the sensing ability of **1′**, all the fluorescence emission spectral data were collected by utilizing a HORIBA JOBIN YVON Fluoromax-4 spectrofluorometer. For the fluorescence experiments, the excitation and emission wavelengths were chosen to be 334 and 434 nm, respectively.

Preparation of 10 mM solutions of hydrazine and other analytes. For the fluorescence detection of hydrazine, 10 mM aqueous solutions of each analyte (hydrazine, arginine, cysteine, serine, glucose, urea, thiourea, hydroxylamine, sodium salts of bisulfate, thiosulfate, sulfide, nitrate, fluoride, chloride, bromide and iodide) were prepared.

Preparation of the HEPES buffer suspension of 1′ (pH = 7.4). 0.2 mg of **1′** was suspended in 2 mL of HEPES buffer (10 mM, pH = 7.4), which was followed by sonication for 15 min. The resulting suspension of **1′** was utilized in all the fluorescence experiments. Here it is worth to mention that 10 mM HEPES buffer (pH = 7.4) solution was prepared by following reported protocol.[2, 3]

Procedure for the fluorescence detection of hydrazine in HEPES buffer (pH = 7.4). All the fluorescence sensing and cellular imaging experiments for hydrazine were performed under biological-like conditions (pH= 7.4). We have used the fixed excitation wavelength of 334 nm during all the fluorescence experiments and emission spectra were collected in the range of 345-620 nm. We have conducted the concentration dependent fluorescence studies using 10 mM aqueous solutions of all analytes. Each analyte was included (45 μL in every addition) to the suspension of **1′** in 10 mM 2 mL HEPES buffer in a quartz cuvette. The fluorescence turn-on behavior of **1′** in HEPES buffer (pH = 7.4) was monitored upon gradual introduction of hydrazine solution. Saturation of the emission intensity was found after the addition of 450 μL of 10 mM solution of hydrazine. For the time dependent studies, 10 mM of hydrazine solution (450 μL) was added to the HEPES suspension of **1′** and the fluorescence spectra were recorded until the saturation point is attainment. The selectivity of **1′** towards hydrazine over other competitive analytes was inspected after the inclusion of competitive analyte solution (450 μL) to the HEPES suspension of **1′**, followed by the addition of hydrazine solution (450 μL) to the mixture. The fluorescence turn-on response of **1′** was measured after the introduction of hydrazine solution.

Cell viability assay. The cellular cytotoxicity of **1′** or hydrazine on MDAMB-231 cells were examined as described previously.[4] Cells were seeded at 10,000 per well in a 96-well plates in 200 μL complete media. Cells were treated with different concentration (0-100 μg/mL) of **1′** or hydrazine (0-100 μM) for 24 h duration in serum free medium at 37 °C. After the mentioned time period, images of cells were captured by Cytell imaging system (GE healthcare). Finally, MTT assay was performed to measure the cellular viability. The viability of the untreated cells was assumed as 100% and used to expressed the viability of the treated cells.

Cellular imaging experiments. The MDAMB-231 cells were cultured in DMEM F12 media containing 10% FBS and 1% antibiotic cocktail as described earlier.[4] The cells were seeded at a density of 25000/well in a 96 well plate. Cells were loaded with probe **1′** (75 μg/mL) for 10 h in serum containing media. In the next, cells were washed with phosphate buffered saline three times in order to remove excess probe and treated with hydrazine (100 μM) for 30 min at 37 °C in phosphate buffered saline. The cells were observed in the bright field and blue channel (λ_{ex} = 334 nm, λ_{em} = 426 nm) using Cytell cell imaging system (GE Healthcare), and images were captured from randomly selected fields.

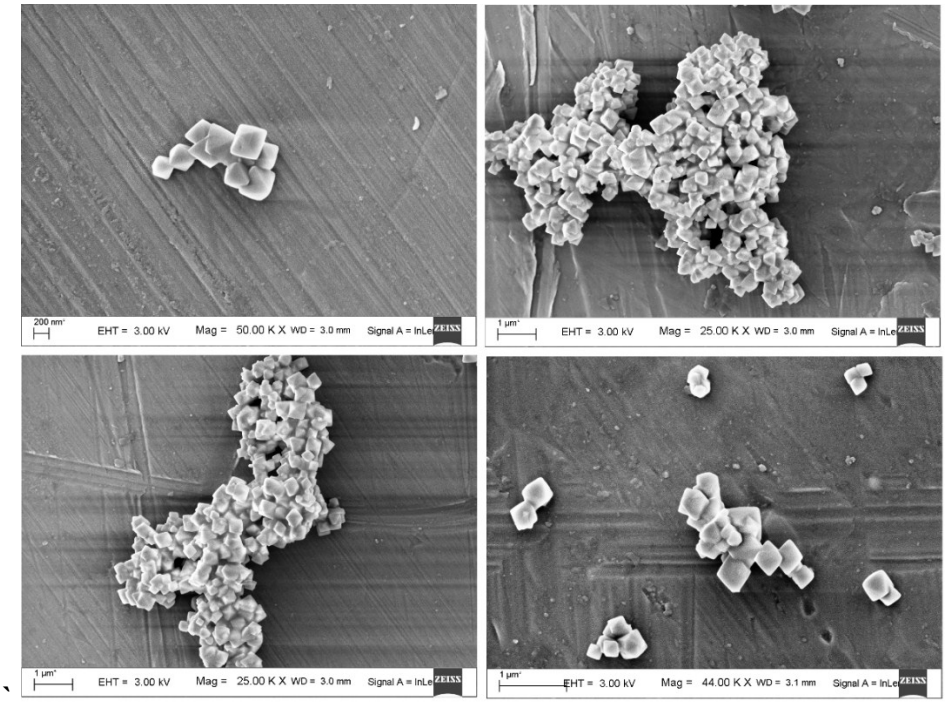

Fig. S1 FE-SEM images of compound **1′**.

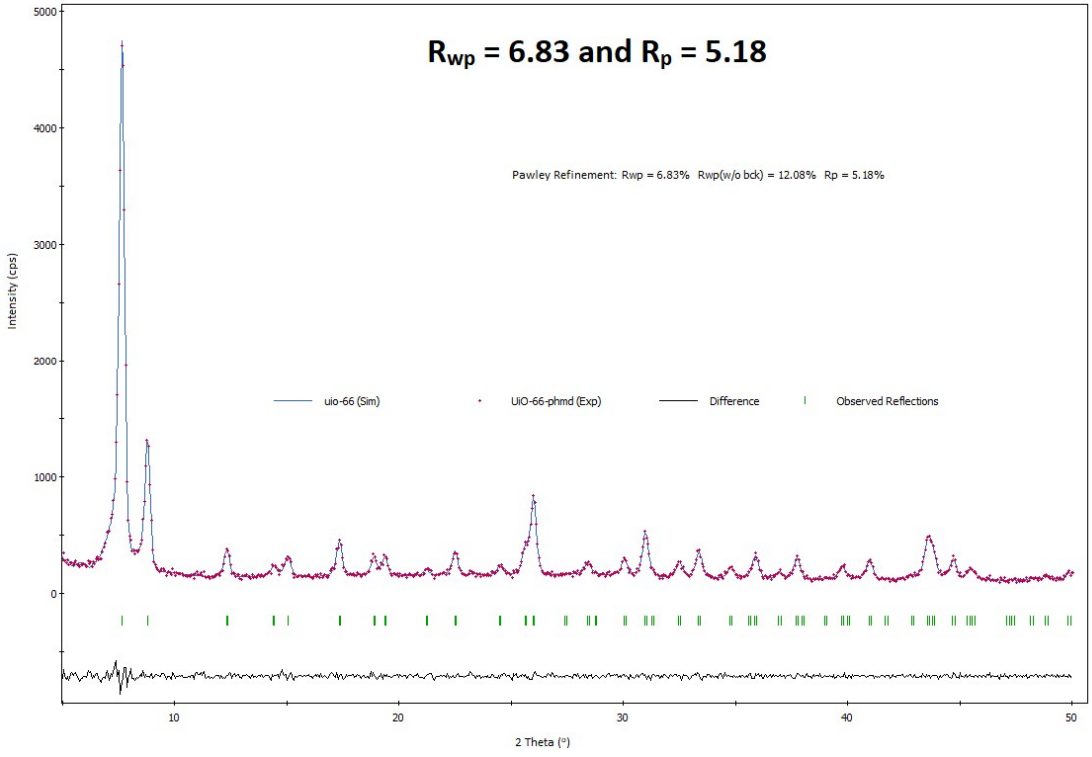

Fig. S2 Pawley refinement for the XRPD pattern of as-synthesized **1**. Red dots and blue lines denote observed and calculated patterns, respectively. The observed reflection and difference plot are displayed at the bottom (R_p = 5.18%, R_{wp} = 6.83%).

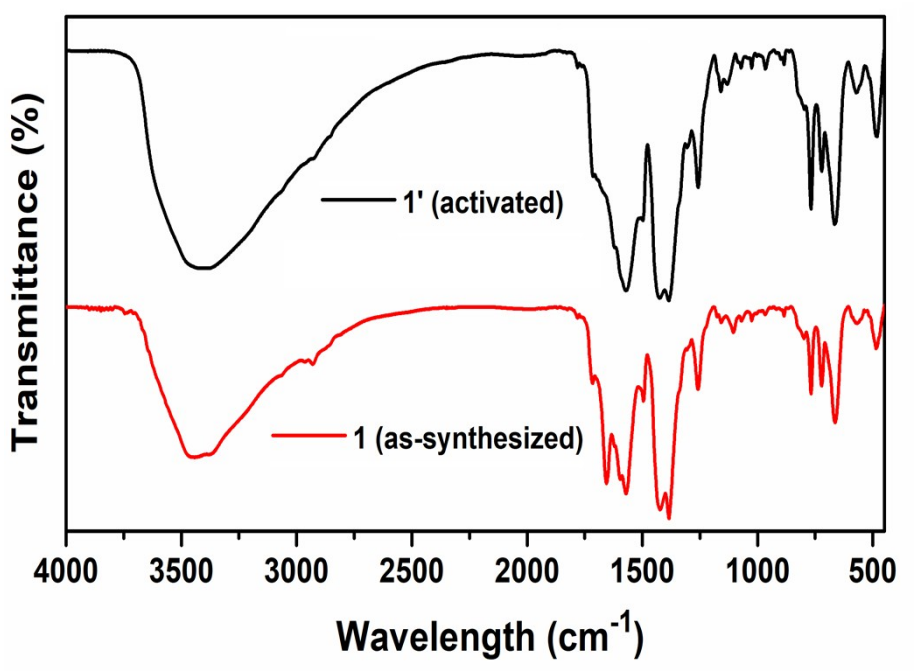

Fig. S3 FT-IR spectra of the as-synthesized (red) and activated (black) forms of compound **1**.

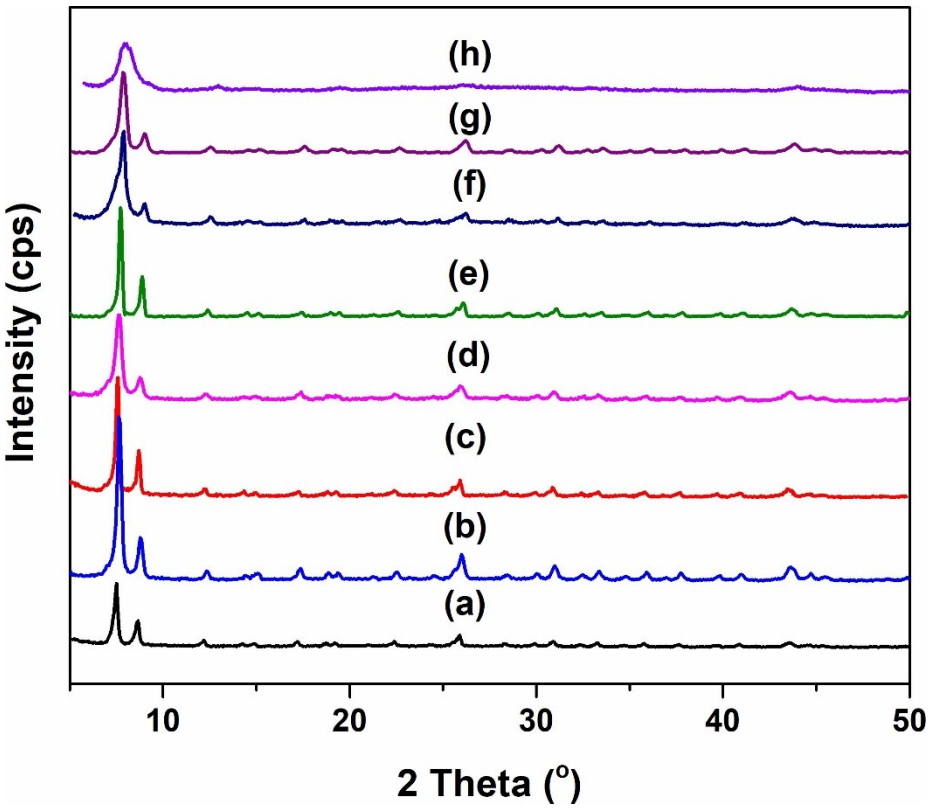

Fig. S4 XRPD patterns of compound **1** in different forms: (a) as-synthesized, (b) activated, (c) after BET measurement, (d) after hydrazine sensing, (e) in water, (f) in acetic acid, (g) in methanol and (h) in 1(M) HCl.

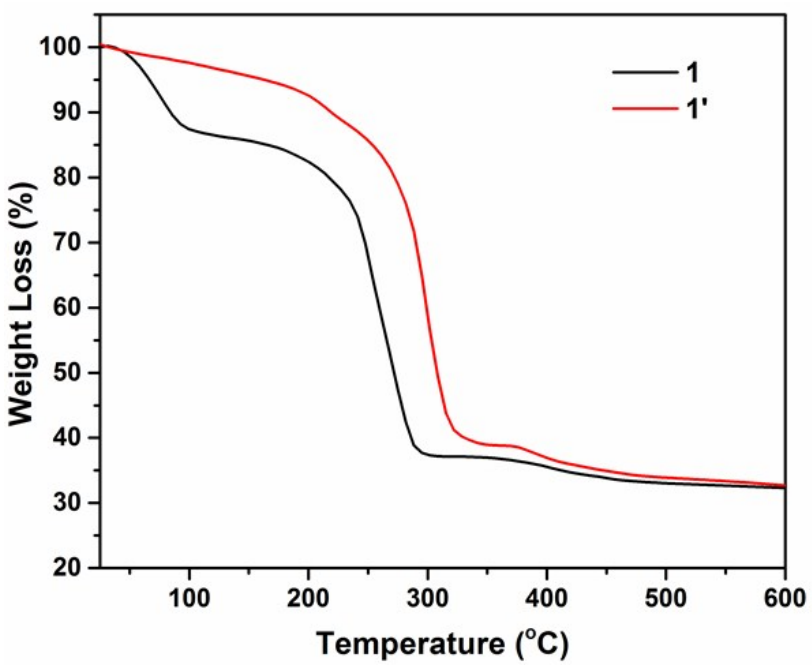

Fig. S5 TG curves of as-synthesized (black) and activated (red) forms of compound **1** measured under N_2 atmosphere with a heating rate of 5 °C min^{-1}.

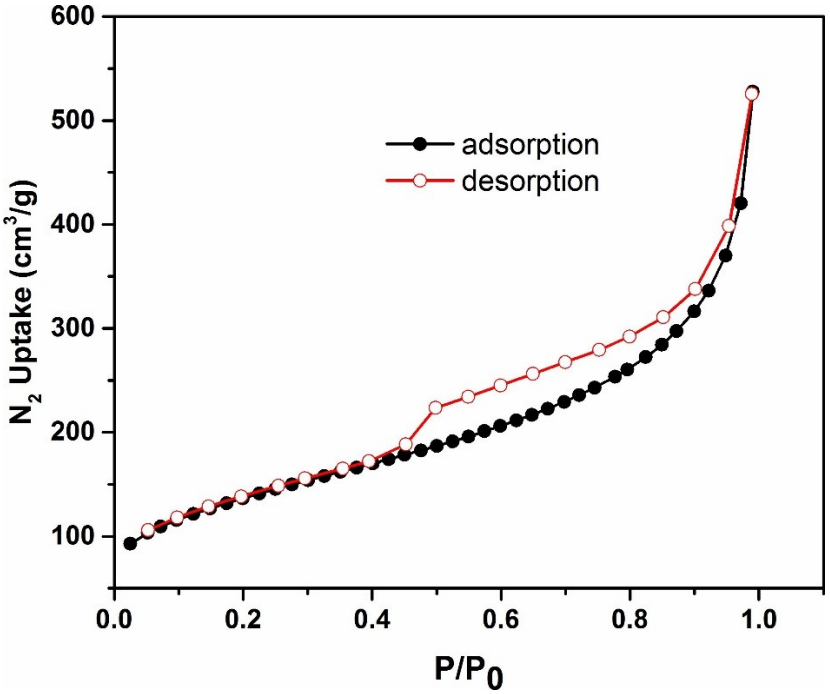

Fig. S6 N_2 adsorption (filled circles; black) and desorption (empty circles; red) isotherms of compound **1′** measured at –196 °C.

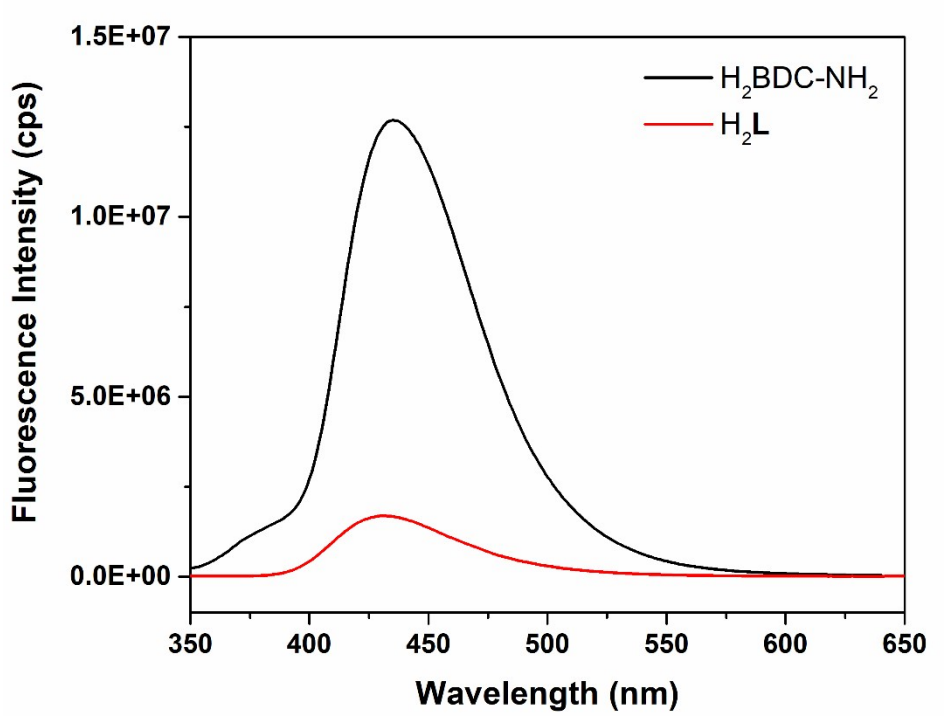

Fig. S7 Fluorescence emission spectra of H_2BDC-NH_2 (black line) and H_2**L** (red line) ligands in DMSO (λ_{ex} = 334 nm and λ_{em} = 434 nm).

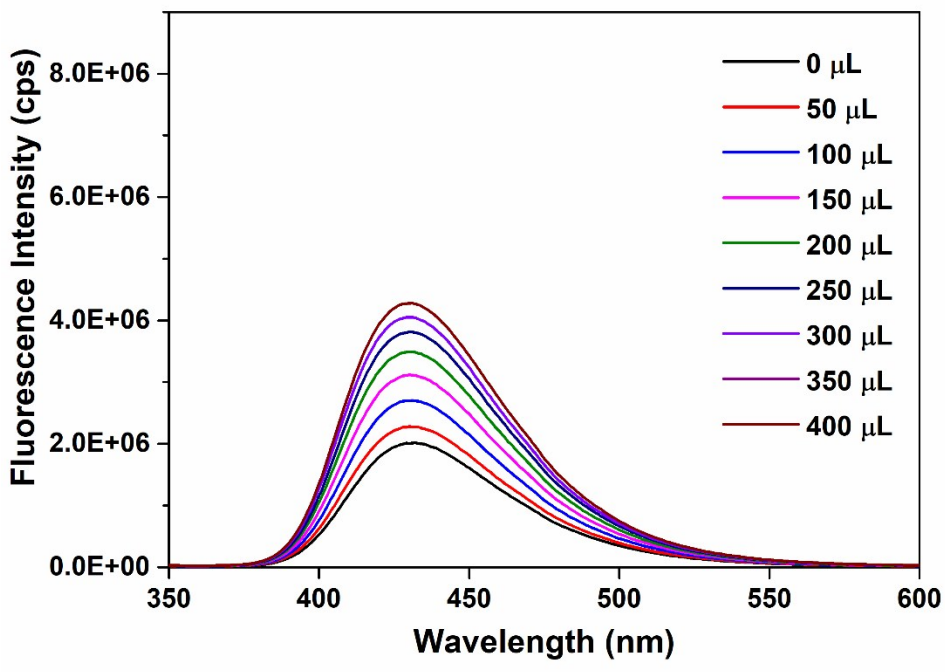

Fig. S8 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM arginine solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

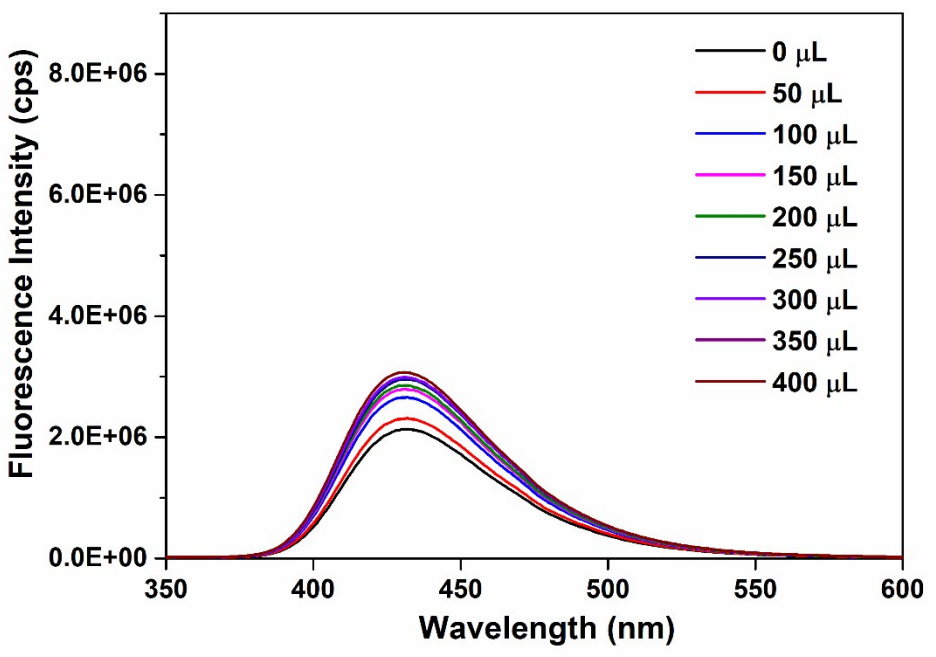

Fig. S9 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM cysteine solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

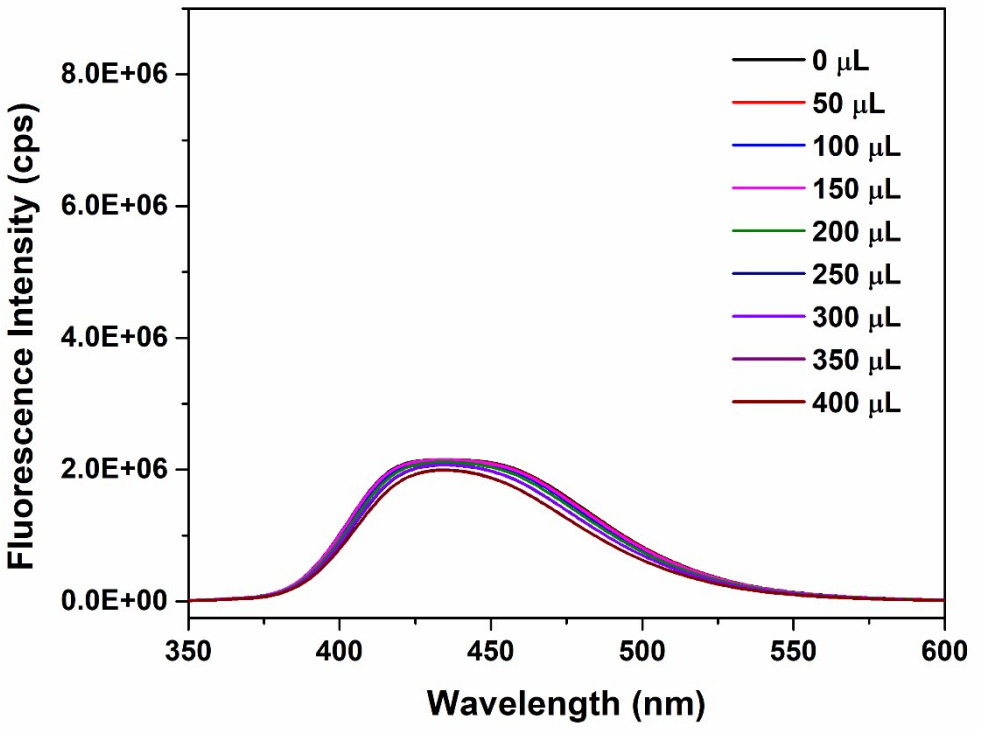

Fig. S10 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM glucose solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

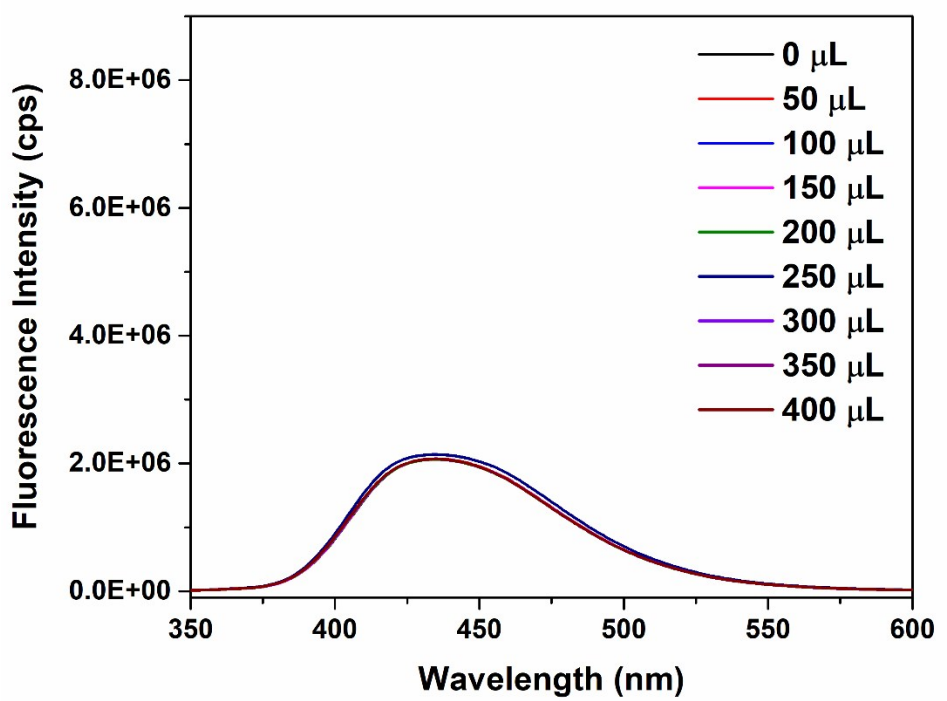

Fig. S11 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM hydroxyl amine solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

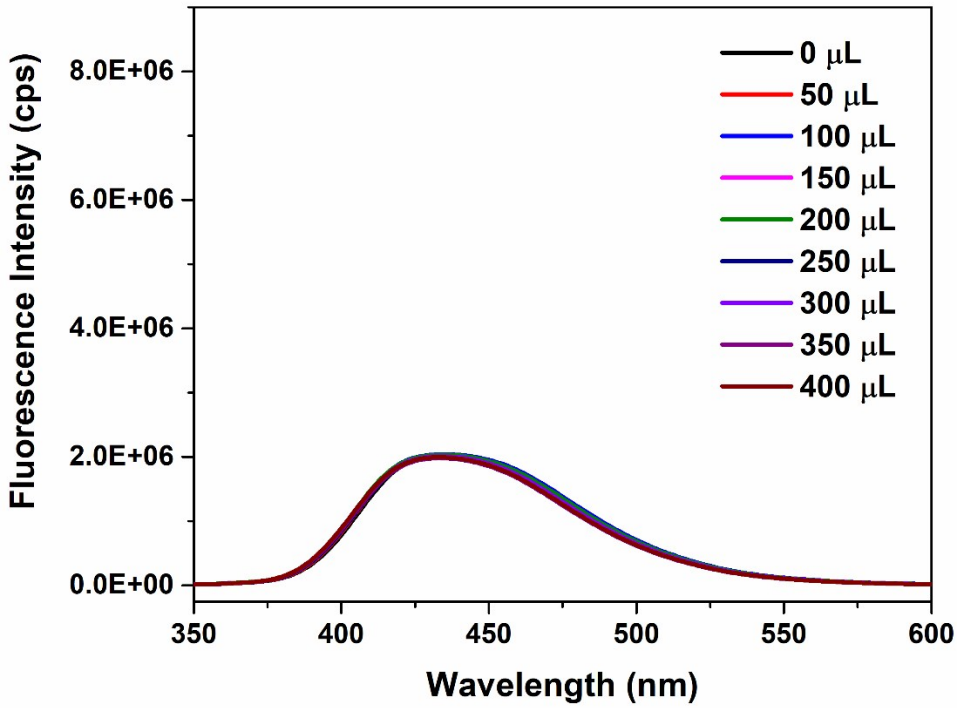

Fig. S12 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM Na_2S solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

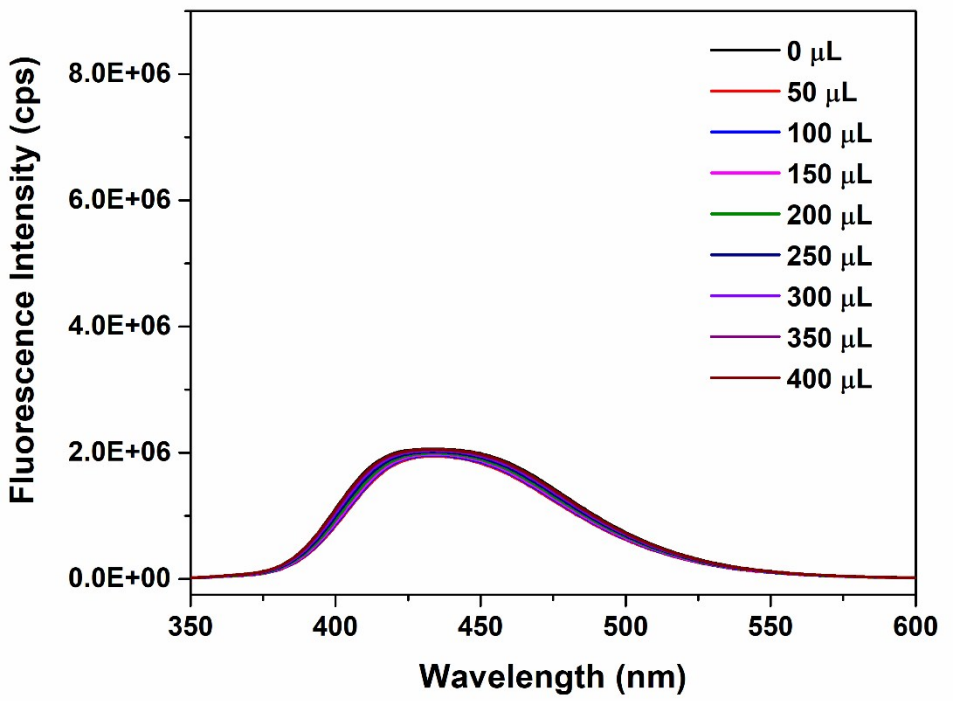

Fig. S13 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM NaBr solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

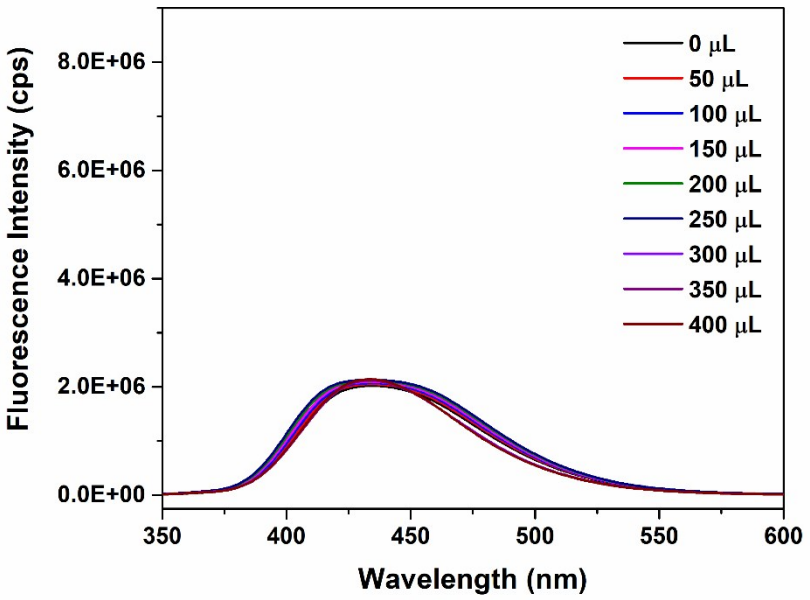

Fig. S14 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM NaCl solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

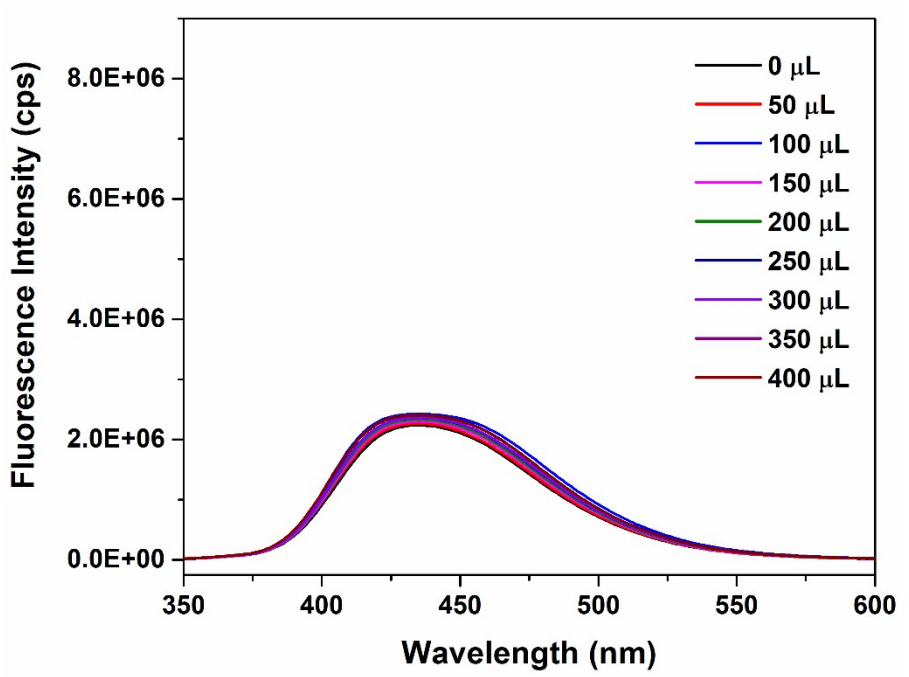

Fig. S15 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM NaF solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

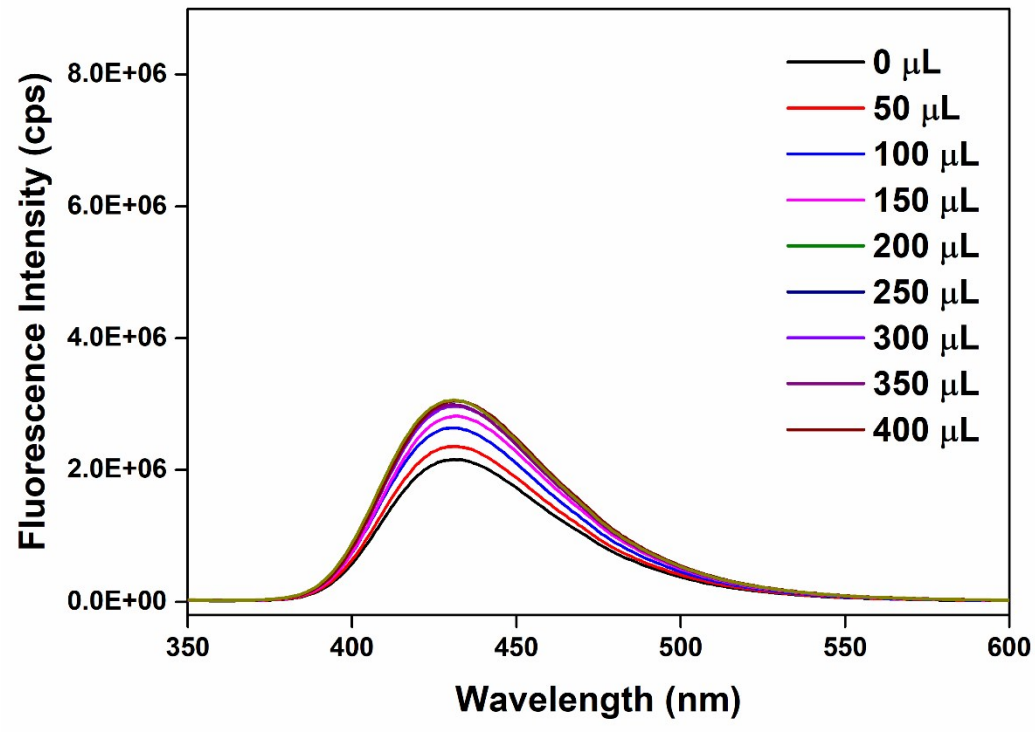

Fig. S16 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM $NaHSO_3$ solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

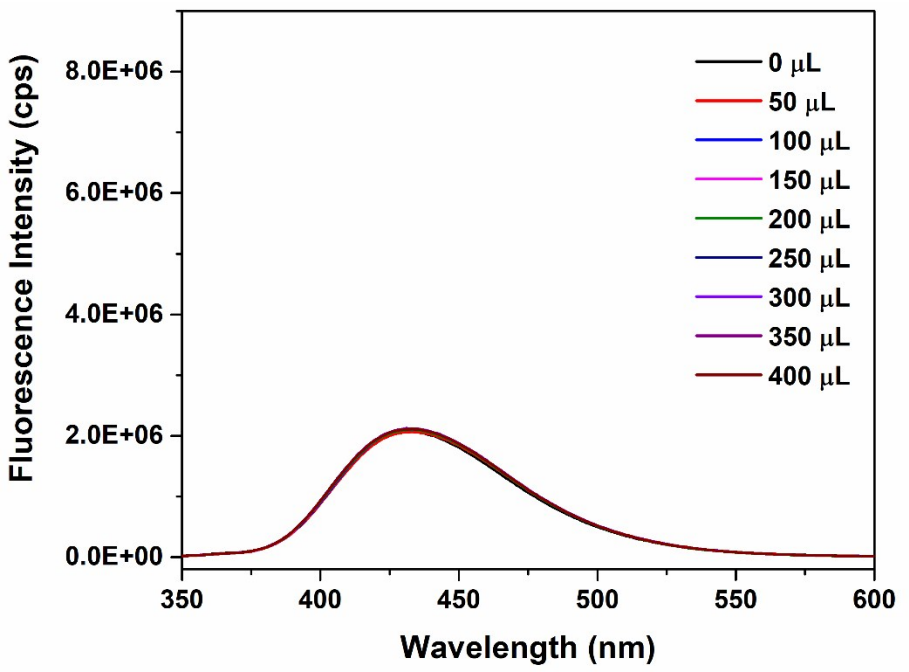

Fig. S17 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM NaI solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

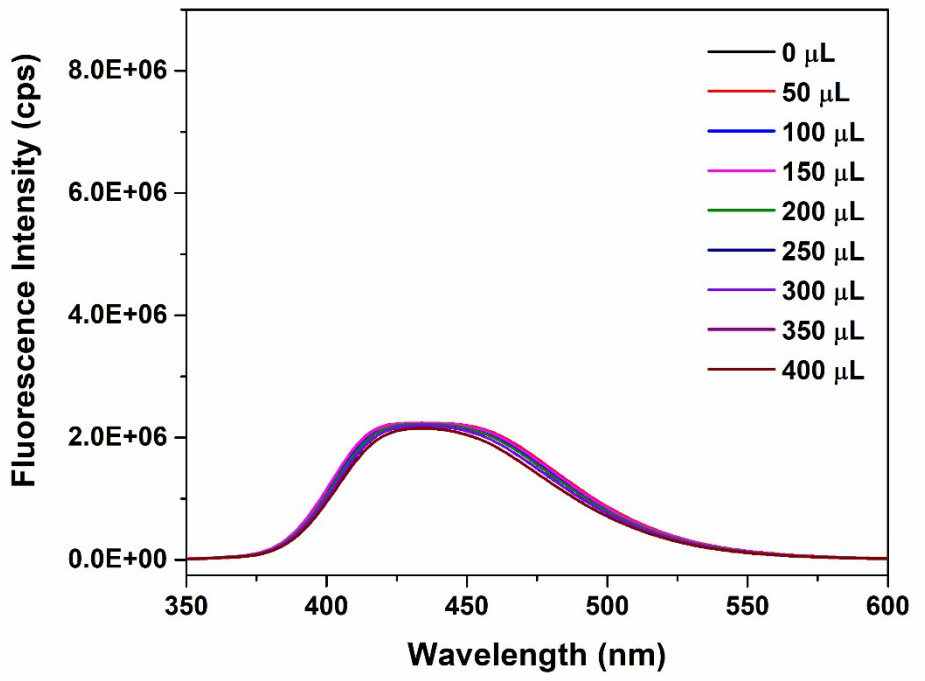

Fig. S18 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM $NaNO_2$ solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

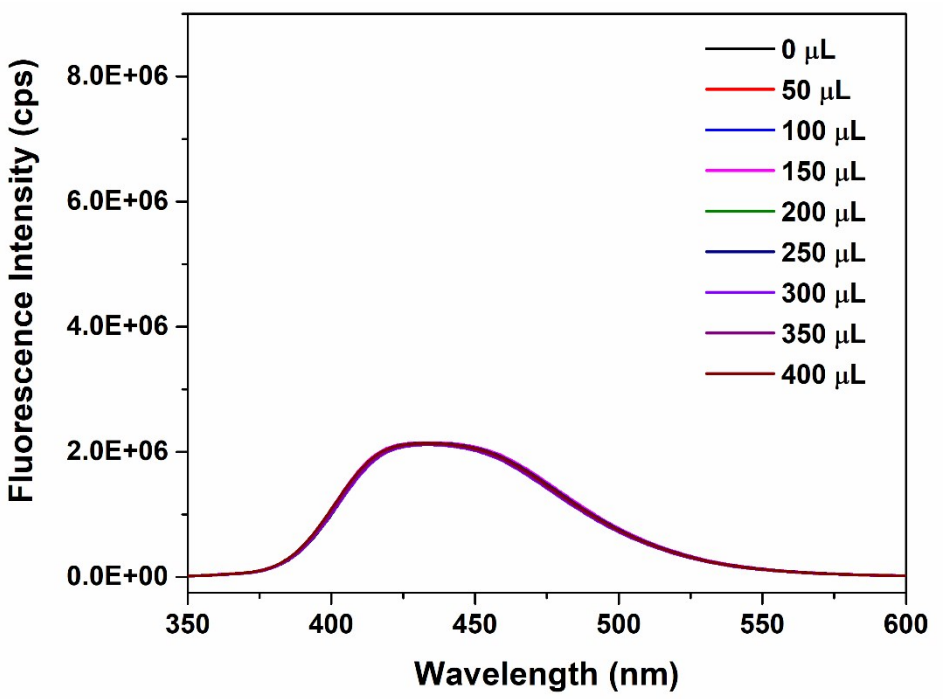

Fig. S19 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM NaSCN solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

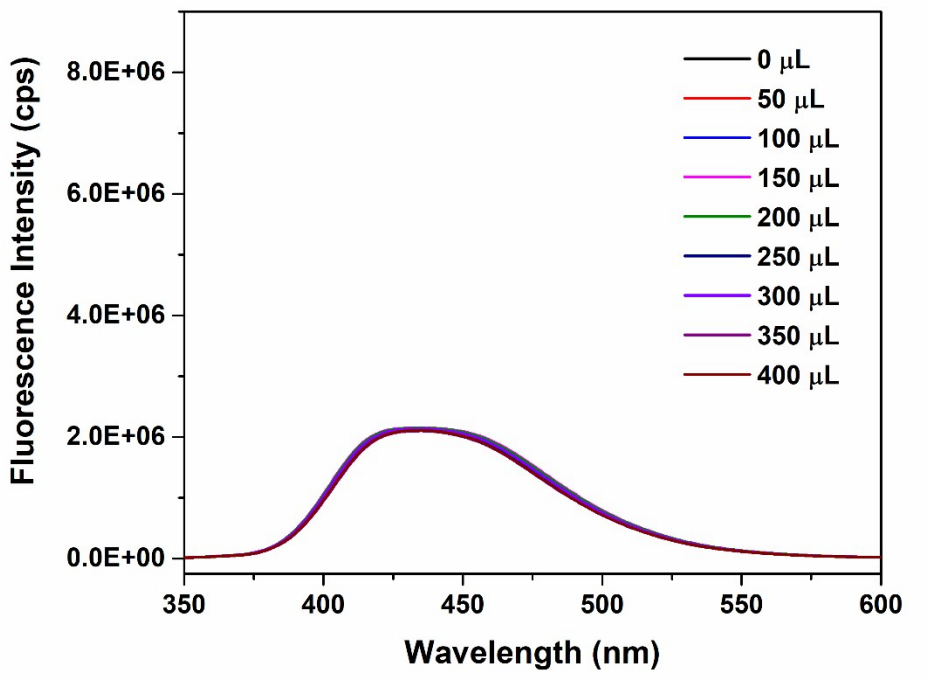

Fig. S20 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM serine solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

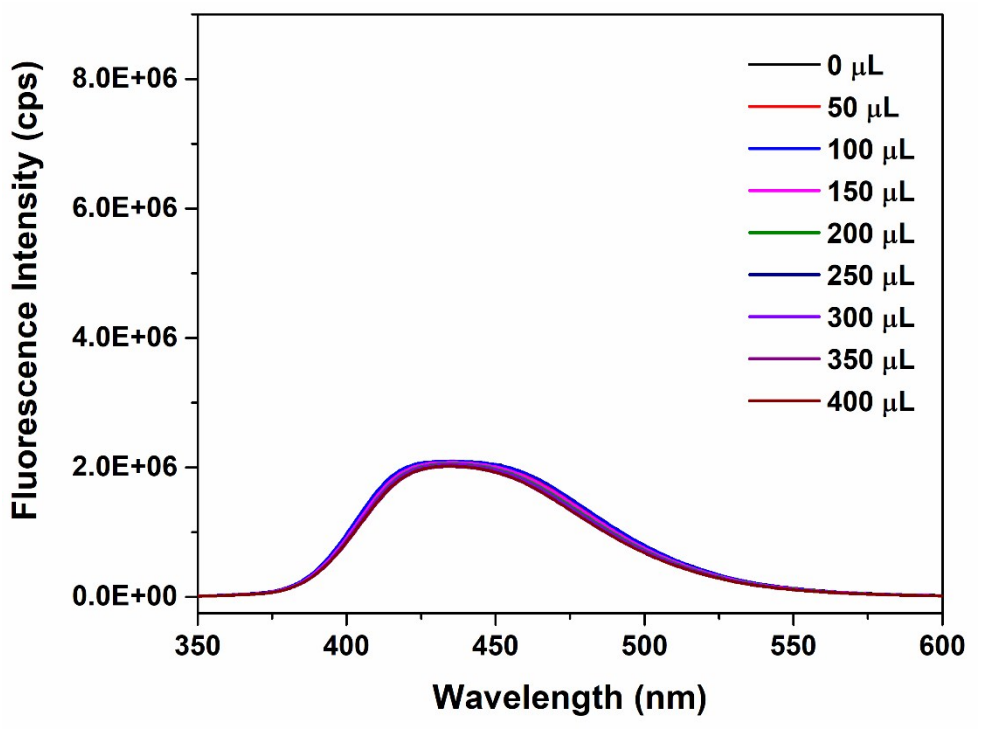

Fig. S21 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM thiosulfate solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

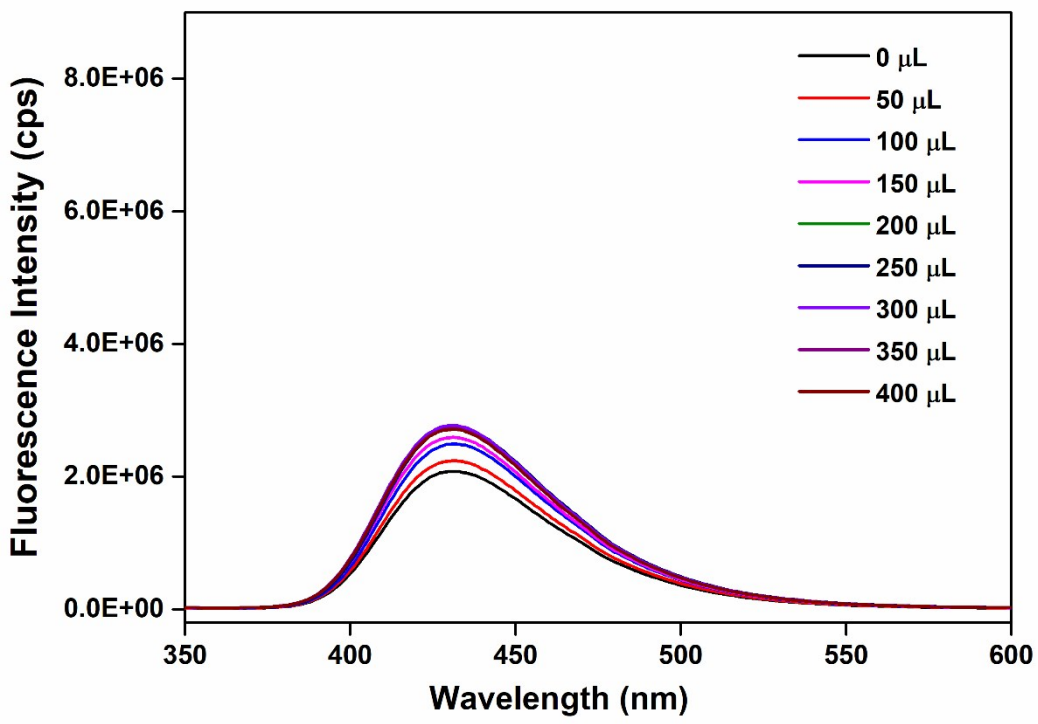

Fig. S22 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM thiourea solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

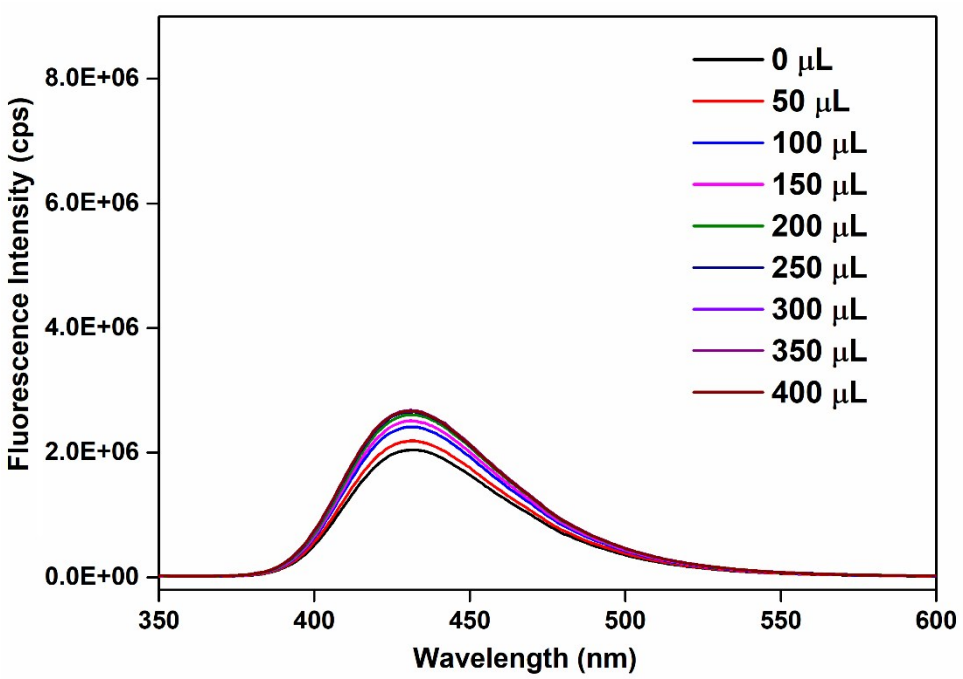

Fig. S23 Change in fluorescence intensity of compound **1′** by incremental addition of 10 mM urea solution to a 2 mL suspension of compound **1′** in 10 mM HEPES buffer (pH=7.4) (λ_{ex} = 334 nm and λ_{em} = 434 nm).

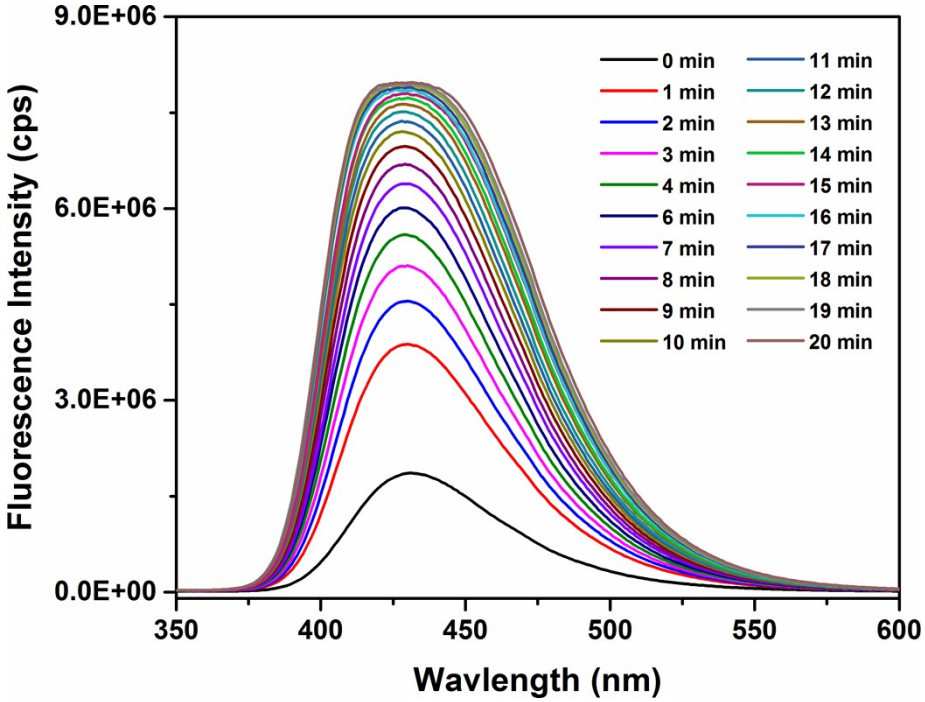

Fig. S24 Change in the fluorescence spectrum of **1′** in presence of 10 mM hydrazine as a function of time (λ_{ex} = 334 nm and λ_{em} = 434 nm).

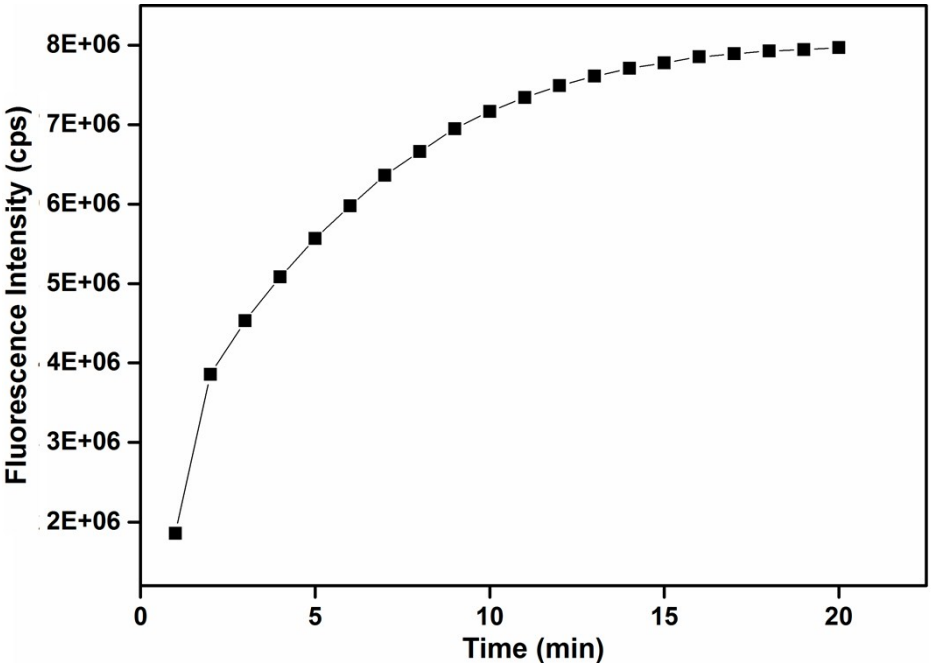

Fig. S25 Change in the fluorescence intensity of **1′** in presence of 10 mM hydrazine as a function of time (λ_{ex} = 334 nm and λ_{em} = 434 nm).

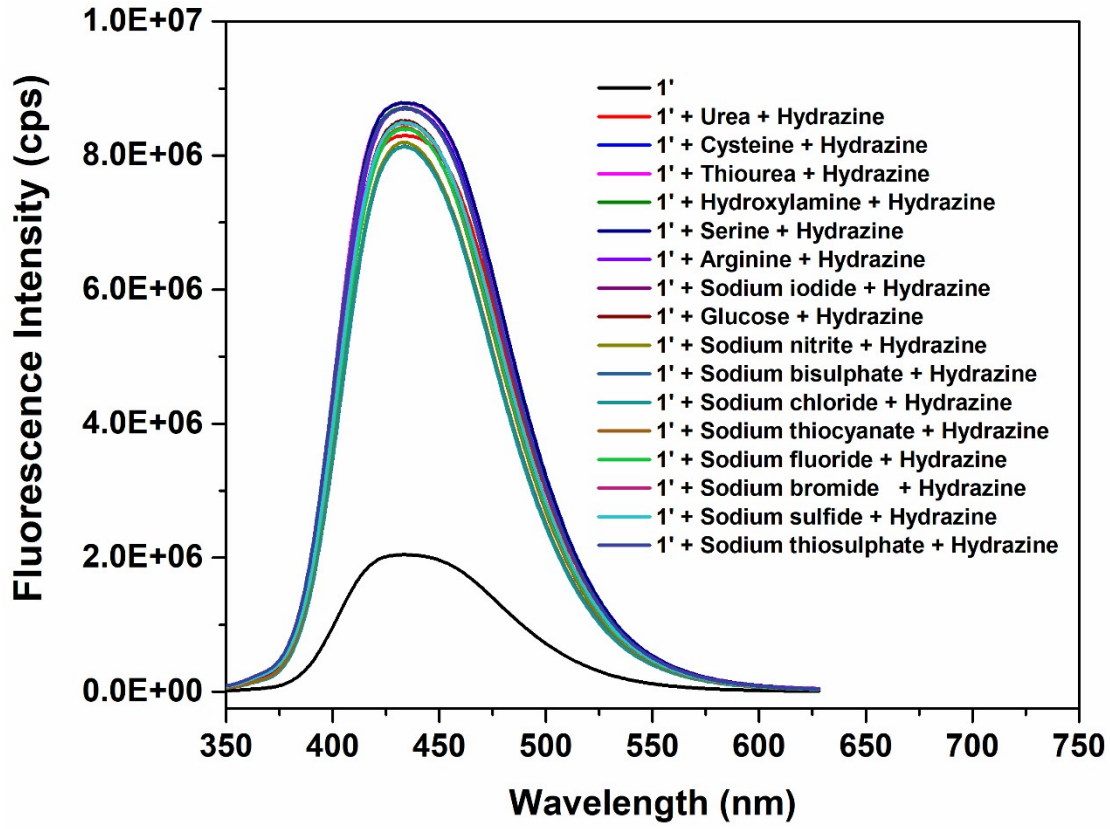

Fig. S26 Fluorescence response of **1′** towards 10 mM hydrazine in presence of 10 mM of different competitive analytes (λ_{ex} = 334 nm and λ_{em} = 434 nm).

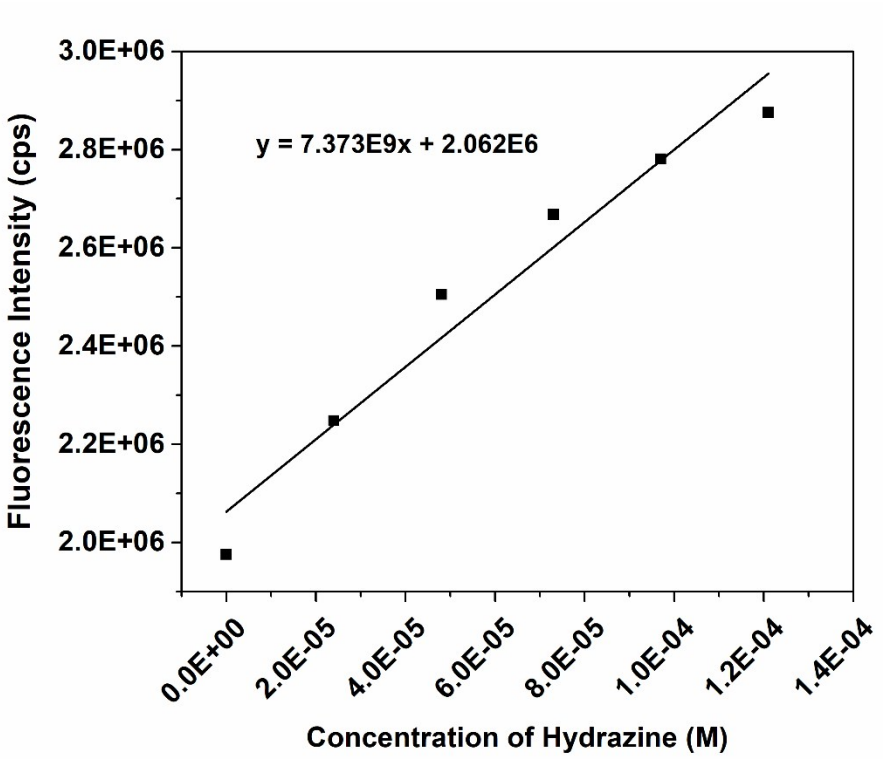

Fig. S27 Change in the fluorescence intensity of **1′** in 10 mM HEPES suspension (pH = 7.4) as a function of hydrazine concentration.

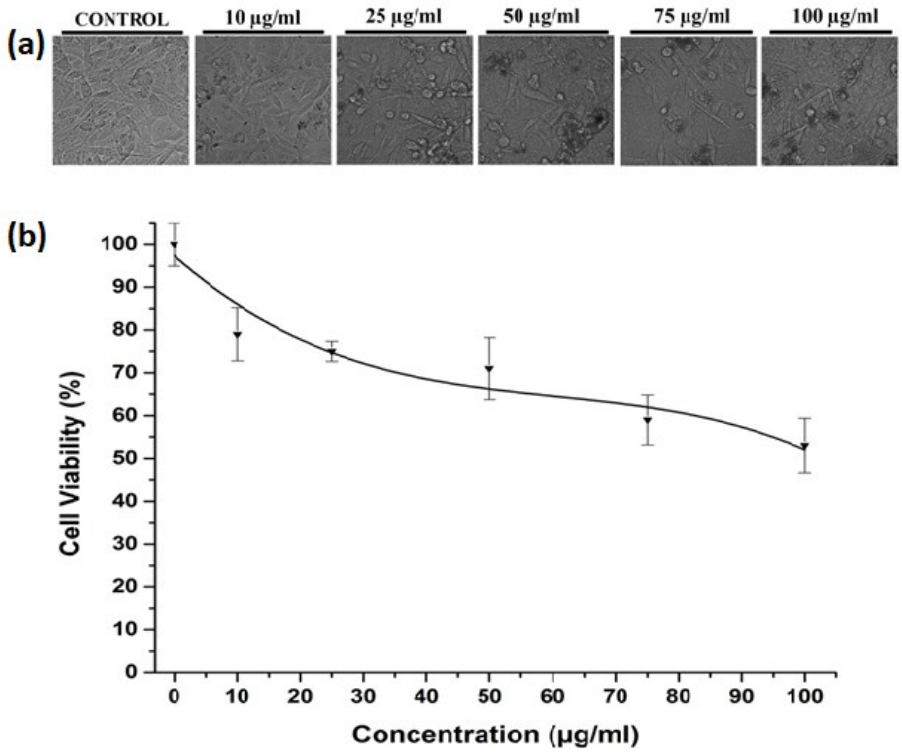

Fig. S28 (a) Morphological analysis of control cells and probe-treated cells. (b) Cell viability assay for probe-treated MDAMB-231 cells.

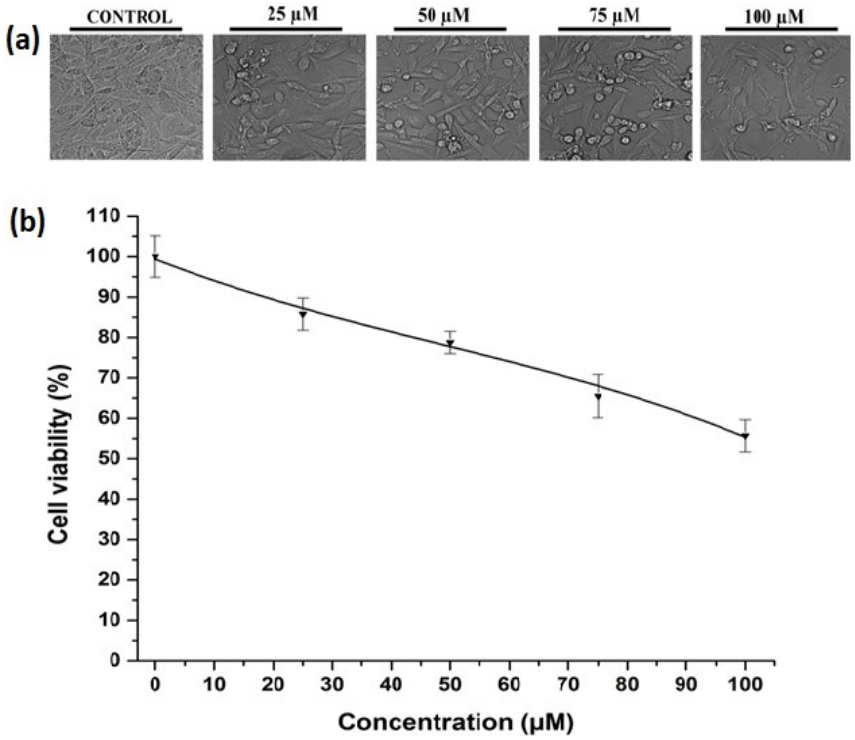

Fig. S29 (a) Morphological analysis of control cells and hydrazine-treated cells. (b) Cell viability assay for hydrazine-treated MDAMB-231 cells.

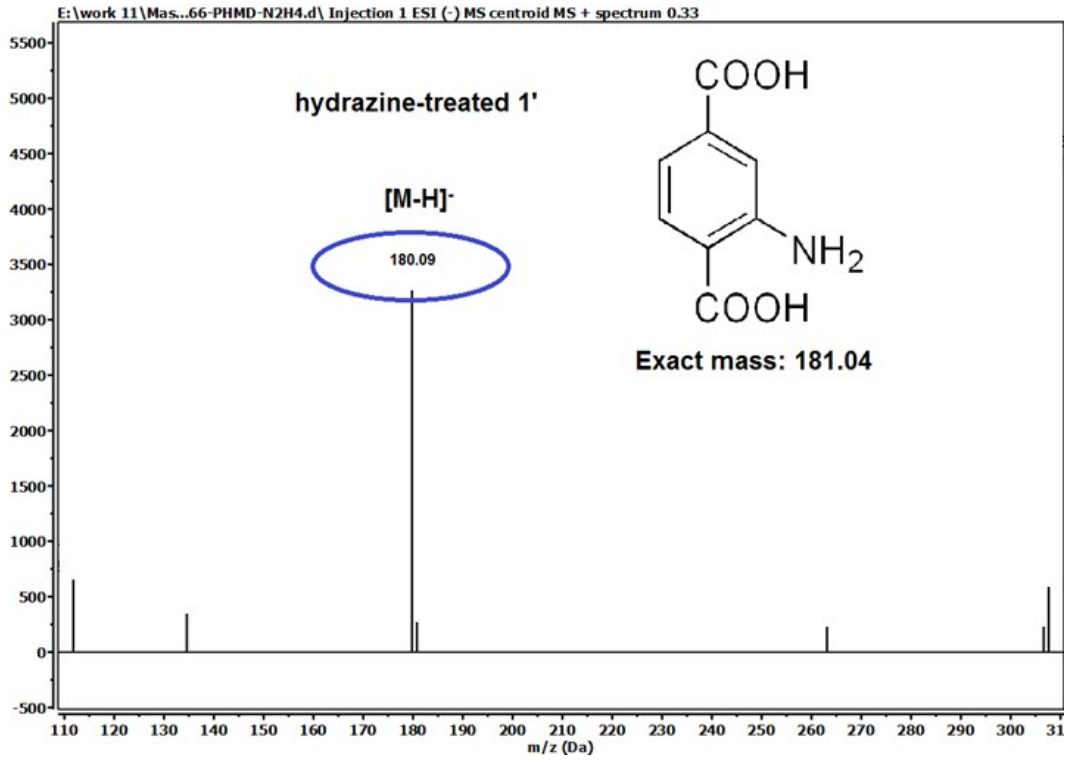

Fig. S30 ESI-MS spectrum of hydrazine–treated **1′** after digestion in HF/MeOH. The spectrum shows m/z peak at 180.09, which corresponds to the $[M-H]^-$ ion (M = mass of $H_2BDC\text{-}NH_2$ ligand).

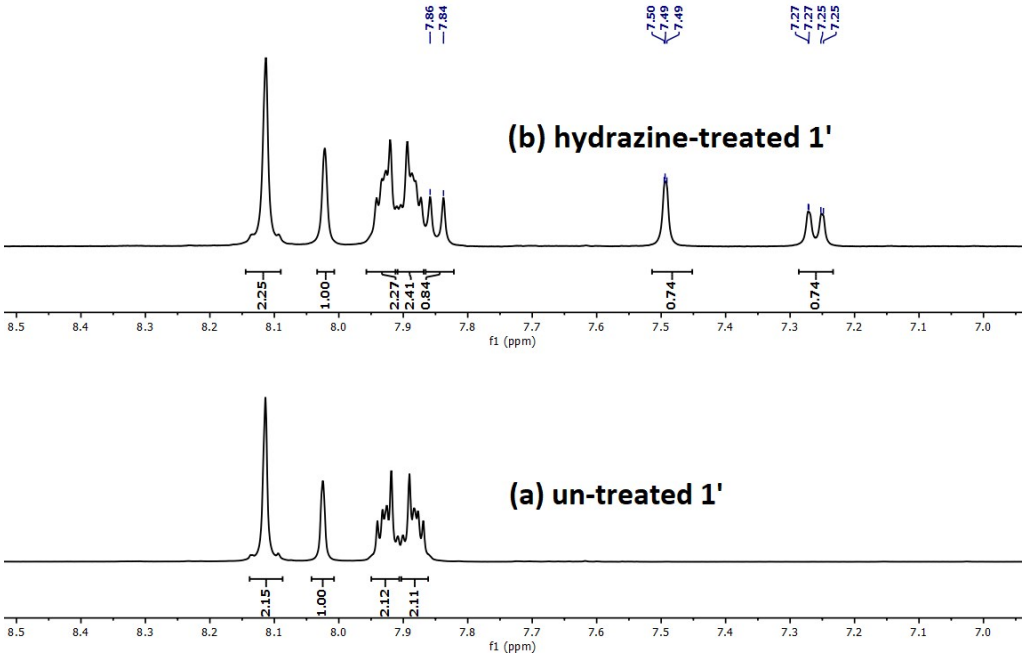

Fig. S31 ^{1}H NMR spectra of (a) un-treated **1′** and (b) hydrazine–treated **1′** after digestion in HF/DMSO-d_6. In the spectrum of hydrazine-treated **1′**, new peaks appear at 7.25, 7.49 and 7.86 ppm, which can be assigned to the aromatic protons of the H_2BDC-NH_2 ligand.

Table S1 Comparison of the sensing performances of various hydrazine sensors.

Sl. No.	Sensor	Sensing Medium	Type of Fluorescence	Detection Limit	Response Time	Ref.
1	$[Zr_6O_4(OH)_4(C_{16}H_7NO_6)_6]$ (**1′**)	HEPES buffer	turn-on	0.87 µM	18 min	this work
2	BTI	HEPES buffer	turn-on	2.9 ppb	20 min	5
3	HyP-1	PBS buffer	turn-on	0.035 ppb	1 h	6
4	P1	PBS buffer	ICT	1.79 nM	40 s	7
5	BPB	HEPES buffer	turn-off	1.87 µM	-	8
6	Naphsulf-O	PBS buffer	turn-on	22 nM	40 min	9
7	BBHC	PBS	turn-on	0.43 µM	1 min	10

		buffer				
8	CFAc	PBS buffer	FRET	0.0474 μM	-	11
9	BI-E	PBS buffer	turn-on	0.057 μM	1 min	12
10	NA-N_2H_4	HEPES buffer	ICT	9.4 nM	15 min	13
11	TAPHP	HEPES buffer	ICT	0.3 μM	60 min	14
12	AB-NDI	DMSO	turn-on	-	-	15
13	TNQ	PBS buffer	ICT	-	-	16
14	HBTM	PBS buffer	turn-on	29 μM	55 min	17
15	NAC	HEPES buffer	turn-on	4.5 μM	4 min	18

References:

1. D. Singh, P. K. Bhattacharyya and J. B. Baruah, *Cryst. Growth Des.*, 2010, **10**, 348–356.
2. A. Buragohain and S. Biswas, *CrystEngComm*, 2016, **18**, 4374-4381.
3. S. Nandi, S. Banesh, V. Trivedi and S. Biswas, *Analyst*, 2018, **143**, 1482-1491.
4. S. J. Deka, A. Roy, V. Ramakrishnan, D. Manna and V. Trivedi, *Chem Biol Drug Des.*, 2017, **89**, 953-963.
5. R. Maji, A. K. Mahapatra, K. Maiti, S. Mondal, S. S. Ali, P. Sahoo, S. Mandal, M. R. Uddin, S. Goswami, C. K. Quah and H.-K. Fun, *RSC Adv.*, 2016, **6**, 70855–77086.
6. Y. Jung, I. G. Ju, Y. H. Choe, Y. Kim, S. Park, Y.-M. Hyun, M. S. Oh and D. Kim, *ACS Sens.*, 2019, **4**, 441–449.
7. Shweta, A. Kumar, Neeraj, S. K. Asthana, A. Prakash, J. K. Roy, I. Tiwari and K. K. Upadhyay, *RSC Adv.*, 2016, **6**, 94959–99496.
8. A. K. Mahapatra, R. Maji, K. Maiti, S. K. Manna, S. Mondal, S. S. Ali, S. Manna, P. Sahoo, S. Mandal, M. R. Uddin and D. Mandal, *RSC Adv.*, 2015, **5**, 58228-58236.
9. W. Chen, W. Liu, X.-J. Liu, Y.-Q. Kuang, R.-Q. Yu and J.-H. Jiang, *Talanta*, 2017, **162**, 225-231.
10. S. Paul, R. Nandi, K. Ghoshal, M. Bhattacharyy and D. K. Maiti, *NewJ. Chem.*, 2019, **43**, 3303-3330.
11. Z. Zhang, Z. Zhuang, L. L. Song, X. Lin, S. Zhang, G. Zheng and F. Zhan, *J. Photochem. Photobiol., A*, 2018, **358**, 10-16.
12. J. Ma, J. Fan, H. Li, Q. Yao, J. Xia, J. Wang and X. Peng, *Dyes Pigm.*, 2017, **138**, 39-46.

13. X. Xia, F. Zeng, P. Zhang, J. Lyu, Y. Huang and S. Wu, *Sens. Actuators, B*, 2017, **227**, 411-418.
14. Z. Luo, B. Liu, T. Qin, K. Zhu, C. Zhao, C. Pan and L. Eang, *Sens. Actuators, B*, 2018, **263**, 229-236.
15. D. Zhou, Y. Wang, J. JIa, W. Yu, B. Qu, X. Li and X. Sun, *Chem. Commun.*, 2015, **51**, 10656-10659.
16. S. Yu, S. Wang, H. Yu, Y. Feng, S. Zhang, M. Zhu, H. Yin and X. Meng, *Sens. Actuators, B*, 2015, **220**, 1338-1345.
17. Z. Chen, X. Zhong, W. Qu, T. Shi, H. Liu, H. He, X. Zhang and S. Wang, *Tetrahedron Lett.*, 2017, **58**, 2596-2601.
18. S. Goswami, A. K. Das, U. Saha, S. Maity, K. Khanra and N. Bhattacharyya, *Org. Biomol. Chem.*, 2015, **13**, 2134-2139.