Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

An ICT-based Ratiometric Probe for Hydrazine and Its Application in Live cells

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CONTENTS:

1. Materials and instruments

All solvents used were of analytical grade without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer, using TMS as an internal standard. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. UV-visible spectra were collected on a Perkin Elmer Lambda 35 UV-Vis spectrophotometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse Fluorescence Spectrophotometer (Serial No. FL1109-M018) with slit widths were set at 10 nm and 10 nm for excitation and emission, respectively.

2. Cell incubation and fluorescence imaging

HeLa cells were seeded onto the cover slips at a concentration of 2×10^{4} cells•mL^{-1} and cultured in DMEM in an incubator (37^{o}C, 5% CO_2 and 20% O_2). After 24 hours, the cover slips were rinsed slightly 3 times with PBS to remove the media and then cultured in PBS for later use. In respect to the verification procedure, 5 μM of probe **1** was added to above cellular samples and incubated for 30 min, then the samples were slightly rinsed 3 times with PBS and observed under a Olympus FV1000-IX81 confocal fluorescence microscope, confocal fluorescence image 100× objective lens. And then the cells were incubated with hydrazine (25 μM) in the medium for another 2 hours, then the samples were slightly rinsed 3 times with PBS and observed under the confocal fluorescent microscope to get pictures with white light and fluorescence, respectively.

3. Determination of the detection limit

The detection limit was calculated based on the method reported in the previous literature [S1]. The fluorescence emission spectrum of **1** was measured by three times and the standard deviation of blank measurement was achieved. The fluorescence ratio at 564 nm and 639 nm was plotted as a concentration of hydrazine. The detection limit was calculated by using detection limit = 3σ/k: Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence ratios versus hydrazine concentration.

4. Theoretical and Computational Methods

All the calculations on compound **1** and the corresponding product were carried out with the B3P86/TZVP level of Guassian 09 program[S2] by using density function theory (DFT) and time-dependent density function theory (TD-DFT). Geometries for reactant and product were fully optimized without symmetry constraints. A continuum solvation model, COSMO (conductor-like screening model), is used for the consideration of solvent effects in aqueous solution[S3].

5. Synthetic procedures

Scheme S1. Synthesis of compound

Compound 2 was synthesized according to our previous reported method[S4].

Scheme S2. Synthesis of probe **1**

Synthesis

2-((7-(diethylamino)-2-oxo-2H-benzo[b][1,4]oxazin-3-yl)methylene)malononitrile 1

A solution of 2 (100 mg, 0.41 mmol) and malononitrile (40 mg, 0.61 mmol) in acetonitrile (15 mL) was stirred at 85℃ for about 4 hours. The solvents was dried in vacuo and the crude product was subjected to column chromatography (eluted with n-hexane: ethyl acetate=1/1, v/v) to afford the desired product as a deep purple solid (101 mg, 84% yield). ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 8.03 (s, 1H), 7.64 (d, 1H, J=9.4 Hz), 6.78 (q, 1H, J=2.64 Hz), 6.44 (d, 1H, J=2.64 Hz), 3.56 (q, 4H, J=7.16 Hz), 1.31 (t, 6H, J=7.12 Hz), ^{13}C NMR ($CDCl_3$, 100 MHz), δ 154.08 , 153.20, 151.69, 148.59, 133.40, 132.17, 125.24, 114.58, 112.86, 112.21, 97.04, 82.34, 45.95, 12.66.

TOF-MS: $[M+Na]^+$ 317.1014; found 317.1010.

6. References

[S1] B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du, X. Zhang, *Chem. Commun.* **2011**, 47, 8656-8658.

[S2] The Gaussian 09 package refer to Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Nor-mand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

[S3] A. Klamt, *J. Phys. Chem.* **1996**, 100, 3349-3353.

[S4] M. Hu, J. Fan, H. Li, K. Song, S. Wang, G. Cheng and X. Peng, *Org. Biomol. Chem.* **2011**, 9, 980-983.

7. UV/Fluorescence studies of compound 1

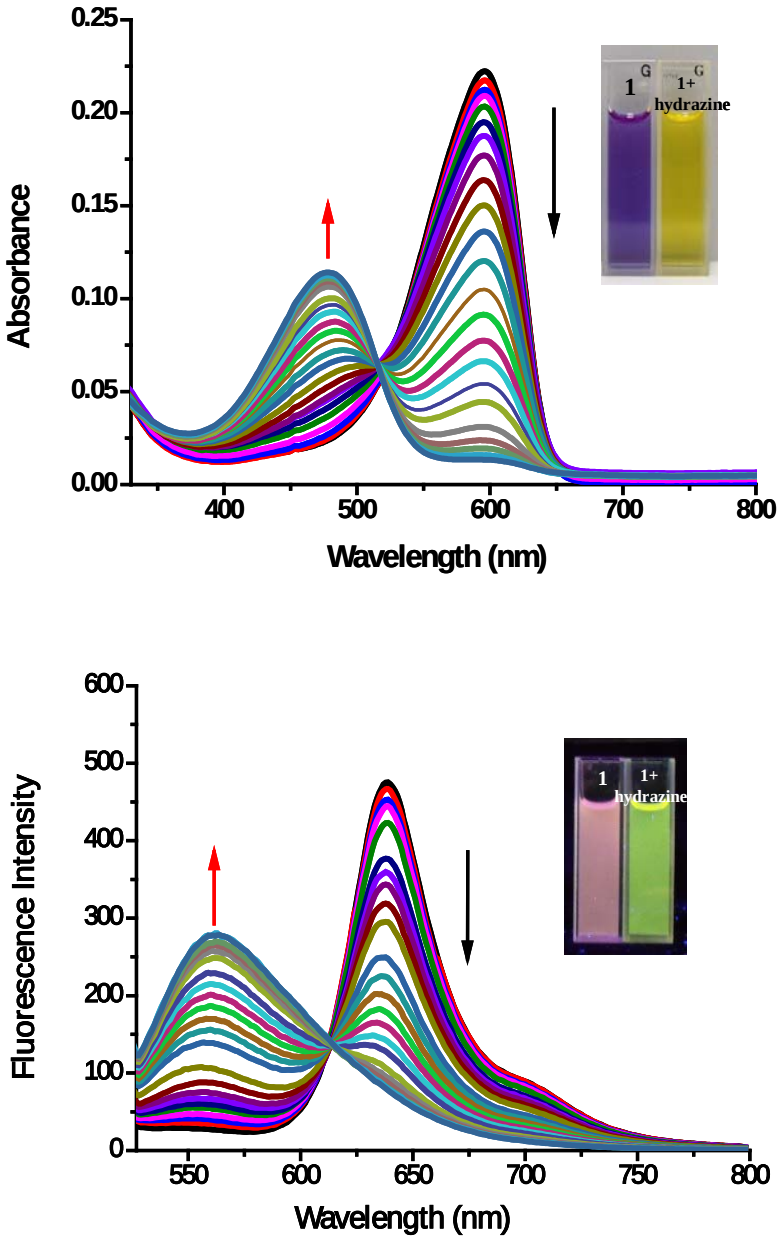


Figure S1. Uv-vis spectra (top) and FL spectra (bottom) of probe **1** in the presence of different concentrations of hydrazine, probe **1** = 5 μM, hydrazine = 0, 0.5, 1.0, 1.5,…..3.5, 5.0, 6.5, 8.0, 9.5,….., 26.0 μM, in a mixture of acetate buffer (pH 3.7, 10 mM) and DMSO (1/9, v/v). Each spectrum was recorded after 20 min. The arrows indicate the change of the absorption and the emission with the increase of hydrazine, λex = 510 nm. The inset shows the color/fluorescence change before and after the addition of hydrazine.

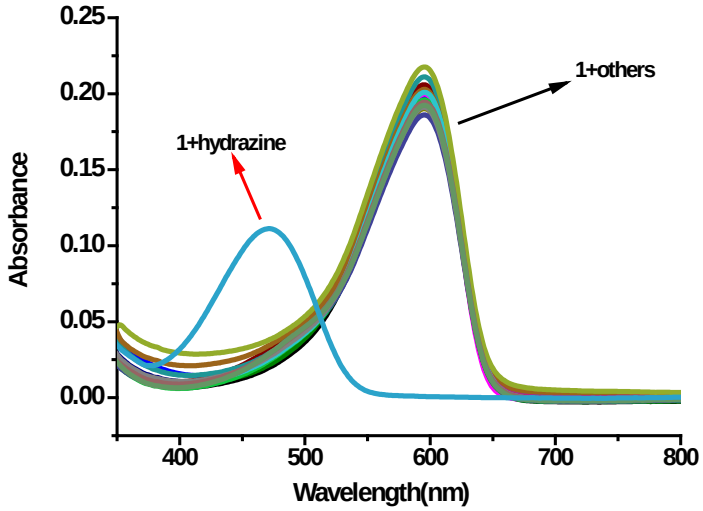


Figure S2. UV-vis spectra of probe **1** in the presence of hydrazine, representative metal ions and anions. probe **1** = 5 μM, hydrazine = 25 μM. 100 μM for Na^{+}, Cl^{-}, Br^{-}, I^{-}, SO_4^{2-}, ClO_4^{-}; 50 μM for Ag^{+}, Cu^{2+}, Co^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, SO_3^{2-}, HCO_3^{-}, SCN^{-}, HPO_4^{2-} In a mixture of acetate buffer (pH 3.7,10 mM) and DMSO (1/9, v/v).Measured after 20 min of mixing.

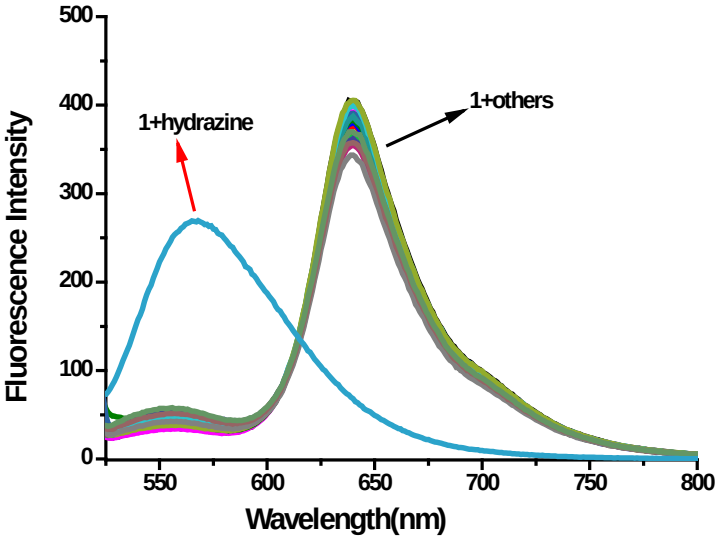


Figure S3. Fluorescence spectra of probe **1** in the presence of hydrazine, representative metal ions and anions. probe **1** = 5 μM, hydrazine = 25 μM. 100 μM for Na^{+}, Cl^{-}, Br^{-}, I^{-}, SO_4^{2-}, ClO_4^{-}; 50 μM for Ag^{+}, Cu^{2+}, Co^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, SO_3^{2-}, HCO_3^{-}, SCN^{-}, HPO_4^{2-} In a mixture of acetate buffer (pH 3.7,10 mM) and DMSO (1/9, v/v).Measured after 20 min of mixing.

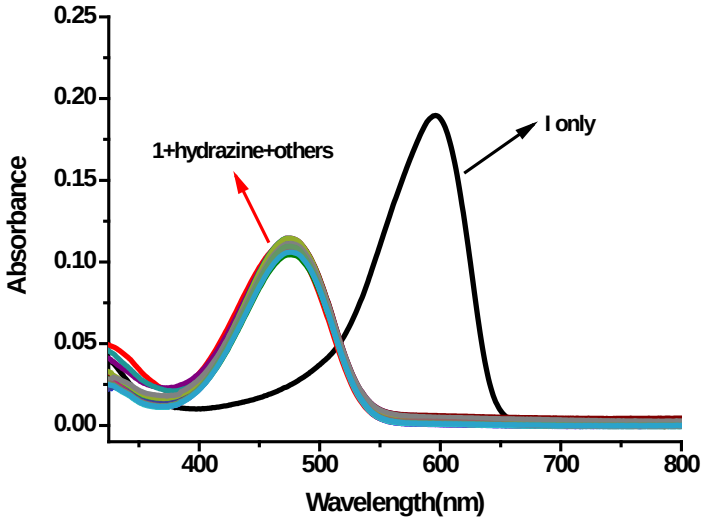


Figure S4. UV-vis spectra of 1-hydrazine system in the presence representative metal ions and anions. probe **1** = 5 μM, hydrazine = 25 μM, 100 μM for Na^{+}, Cl^{-}, Br^{-}, I^{-}, SO_4^{2-}, ClO_4^{-}, 50 μM for Ag^{+}, Cu^{2+}, Co^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, SO_3^{2-}, HCO_3^{-}, SCN^{-}, HPO_4^{2-}.In a mixture of acetate buffer (pH 3.7, 10 mM) and DMSO (1/9, v/v).Measured after 20 min of mixing.

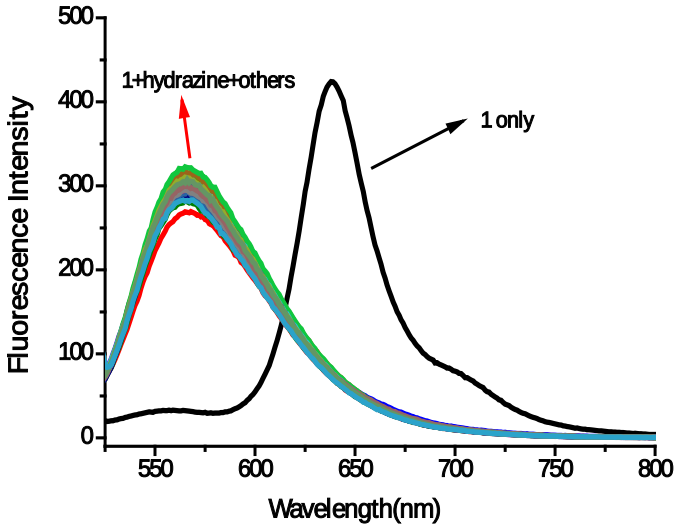


Figure S5. Fluorescence spectra of 1-hydrazine system in the presence representative metal ions and anions. probe **1** = 5 μM, hydrazine = 25 μM, 100 μM for Na^{+}, Cl^{-}, Br^{-}, I^{-}, SO_4^{2-}, ClO_4^{-}, 50 μM for Ag^{+}, Cu^{2+}, Co^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, SO_3^{2-}, HCO_3^{-}, SCN^{-}, HPO_4^{2-}.In a mixture of acetate buffer (pH 3.7, 10 mM) and DMSO (1/9, v/v).Measured after 20 min of mixing.

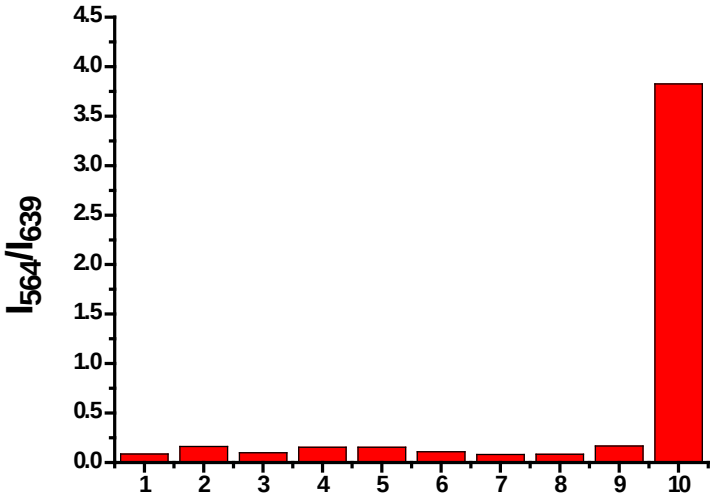


Figure S6. Fluorescence rations responses of probe **1** (5 μM) to various compounds (500 μM for Cys, GSH, thiourea, mercaptonethanol; 50 μM for NH_4^+, lysine, glutamine; 25 μM for $NH_3 \cdot H_2O$) and hydrazine (25 μM) at 564 nm and 639 nm. Conditions: acetate buffer (pH 3.7, 10 mM) and DMSO (1/9, v/v).Each spectrum was recorded after 20 min. λex = 510 nm.1.blank, 2.Cys, 3.GSH, 4.thiourea, 5.mercaptoethanol, 6.lysine, 7.glutamine, 8.NH_4^+, 9.$NH_3 \cdot H_2O$ and 10.hydrazine.

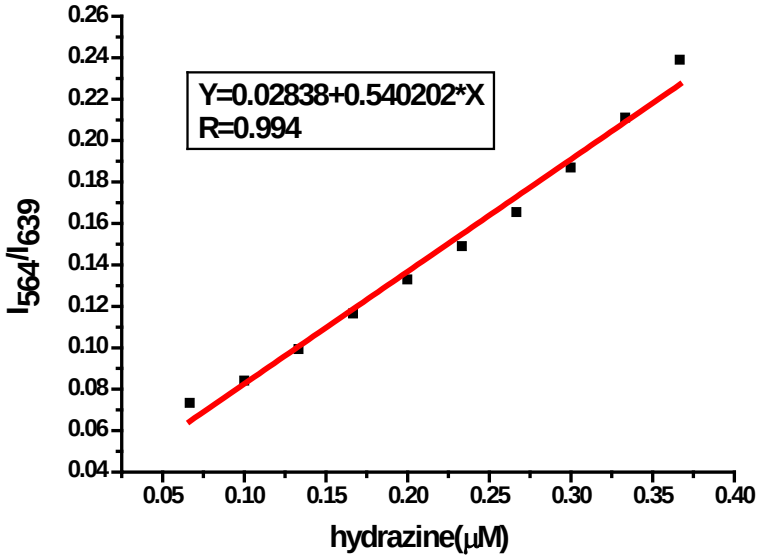


Figure S7. The fluorescence ratios of probe **1** (5 μM) was linearly related to the concentration of hydrazine (0.07–0.37 μM) in DMSO.Y = 0.02838 + 0.54020×X, R = 0.994.

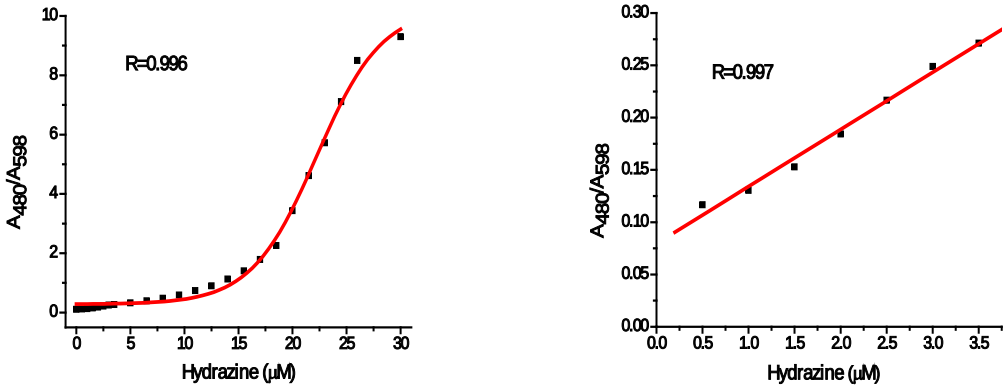


Figure S8. (a) Plot of absorption ratios at 480 nm and 598 nm of probe **1** (5 μM) upon varied concentrations of hydrazine (0–30 μM). **(b)**The absorption ratios of probe **1** (5μM) was linearly related to the concentration of hydrazine (0.5–3.5 μM). In a mixture of acetate buffer (pH 3.7, 10 mM) and DMSO (1/9, v/v). Measured after 20 min of mixing.

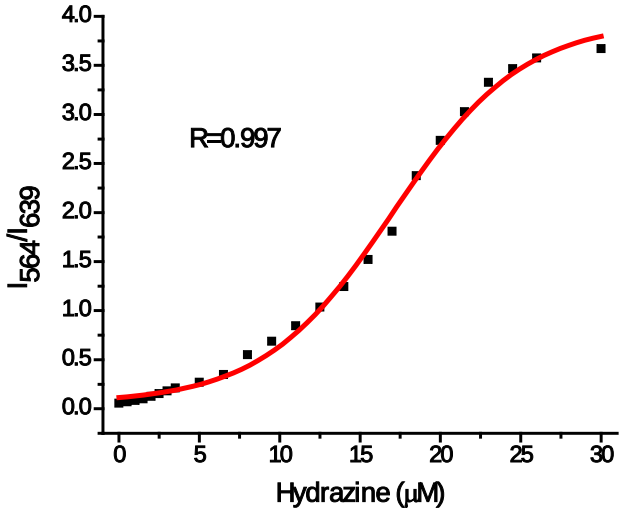


Figure S9. (a) Plot of fluorescence ratios at 564 nm and 639 nm of probe **1** (5 μM) upon varied concentrations of hydrazine (0–30 μM). In a mixture of acetate buffer (pH 3.7, 10 mM) and DMSO (1/9, v/v). Measured after 20 min of mixing.

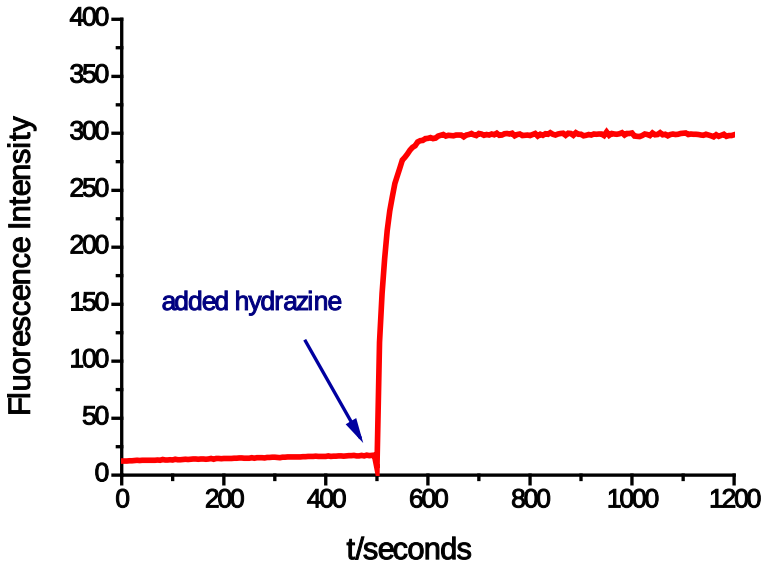


Figure S10. Time-dependent fluorescence changes of **1** with hydrazine. probe **1** = 5 μM, hydrazine = 25 μM. in DMSO. λex = 510 nm, λem = 564 nm.

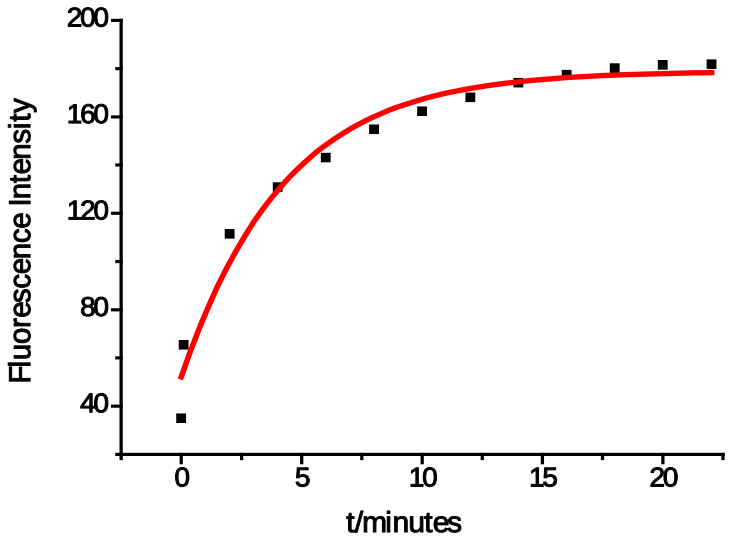


Figure S11. Time-dependent fluorescence changes of **1** with hydrazine. probe **1** = 5 μM, hydrazine = 50 μM in a mixture of acetate buffer (pH 3.7, 10 mM) and DMSO (1/9 v/v). λex = 510 nm, λem = 564 nm.

8. IR spectra of compound 1 and 1 upon addition of hydrazine

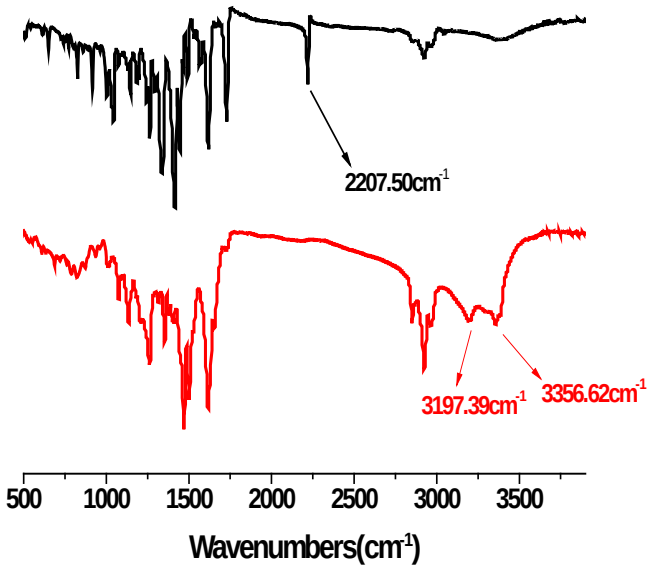


Figure S12. IR spectra of probe **1** (black line), **1** upon addition of hydrazine (red line). **1** showed a sharp band assigned to the C≡N stretching vibrations at 2207.50 cm^{-1}. In the presence of hydrazine, the band disappeared in the product and there emerged two new bands at 3356.62 cm^{-1} and 3197.39 cm^{-1} assigned to the N-H stretching vibration.

9. TOF-MS of 1 upon addition of hydrazine

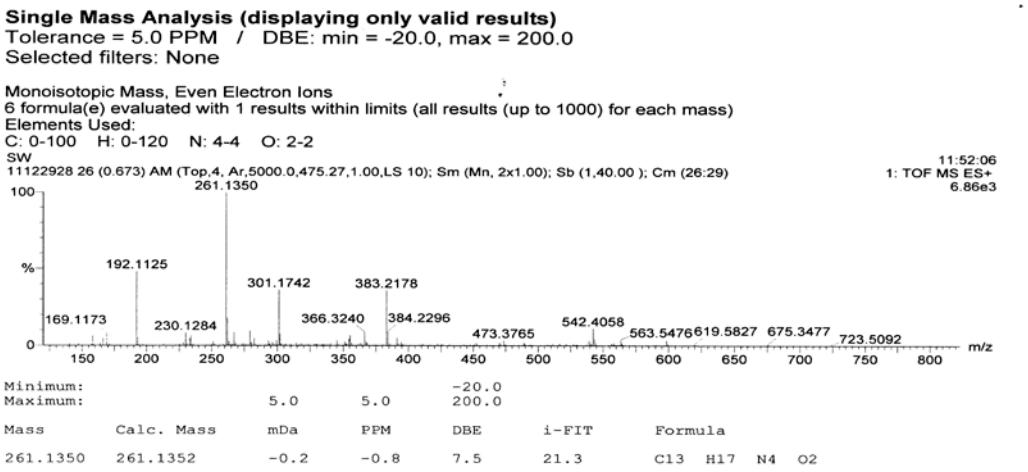


Figure S13. TOF-MS of compound **1** upon addition of hydrazine**.** TOF-MS:$[M+H]^+$ 261.1352;

found 261.1350. The result indicates that the formula of the corresponding compound is $C_{13}H_{16}N_4O_2$.

10. Determinnation of hydrazine in water samples.

Table S1. Determination of hydrazine in water samples

Samples	Hydrazine Added (10^{-7}mol L^{-1})	Proposed method [a]		Electrocatalytic oxidation [b]	
		Found (10^{-7} mol L^{-1})	Recovery (%)	Found (10^{-7} mol L^{-1})	Recovery (%)
Drinking water	10	10.3	103.1	10.2	101.6
	20	20.7	103.5	19.3	98.5
	30	29.6	98.7	31.6	105.1
River water	10	9.7	97.0	9.8	98.0
	20	19.5	97.4	21.2	106.0
	30	31.2	104.1	29.9	99.7

a. Conditions: $I_{564}/I_{639,}$ probe **1** (5 μM), 20 min reaction at room temperature. The samples were first adjust to pH 3.7 acetate buffer and then different concentrations of hydrazine were obtained by diluting the solution into a co-solvent (acetate buffer/DMSO = 1/9).
b. S. Wu, F. Nie, Q. Chen and J. Sun, *Anal. Methods*, **2010**, 2, 1729–1736.

11. Fluorescence images of HeLa cells

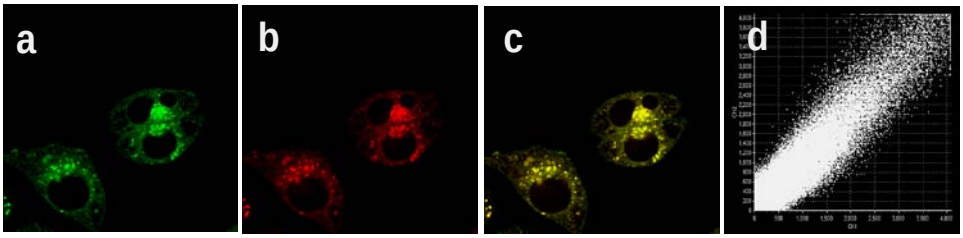


Figure S14. Intracellular distribution of **1** (10 μM) compared with lysosensor green (1 μM). (a) Fluorescence image of lysosensor green; (b) fluorescence image of **1**; (c) merging of a and b; (d) colocalization coefficient of **1** and lysosensor green is 0.9628. The excitation wavelength of lysosensor and probe **1** are 488 nm and 559 nm, respectively.

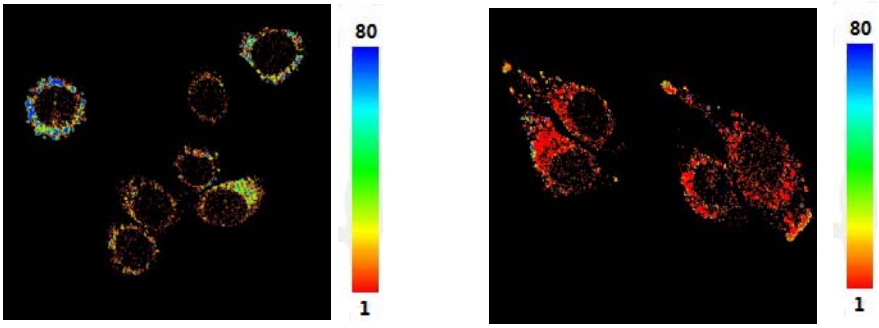


Figure S15. The fluorescence ratio ($F_{620\text{-}660}/F_{520\text{-}560}$) images of HeLa cells stained with probe **1** and hydrazine. (left) Image of cells after treatment with **1** (5 μM) for 0.5 h. (right) Image of cells after treatment with **1** (5 μM) for 0.5 h and subsequent treatment of the cells with 25 μM hydrazine for 2h. The ratiometric images were obtained by the image analysis software of Image Pro-plus 6.0.

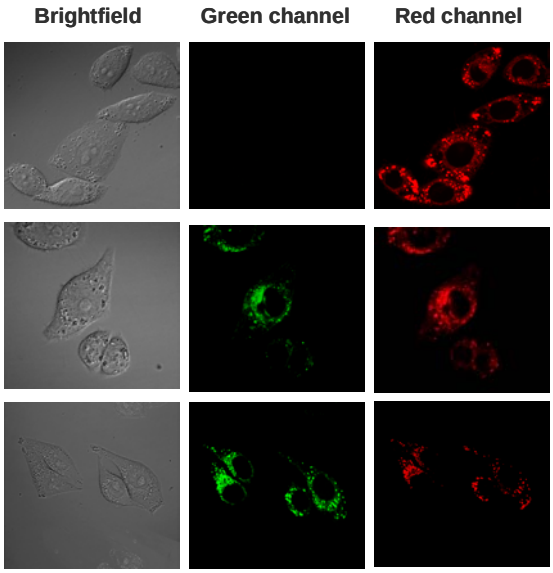


Figure S16. Confocal fluorescence images of HeLa cells. Cells incubated with probe 1 (5 μM) for 0.5 h (top); image of cells after treatment with probe 1 (5 μM) for 0.5 h and subsequent treatment of the cells with 10 μM (middle) and 25 μM (bottom) hydrazine for 2 h. green emission (540 ± 20 nm); red emission (640 ± 20 nm). λex = 515 nm. The figure shows that fluorescence in the green channel obviously increased while the red fluorescence intensity decreased with the increasing of the concentration of hydrazine.

12. Plots and tables of theoretical and computational study

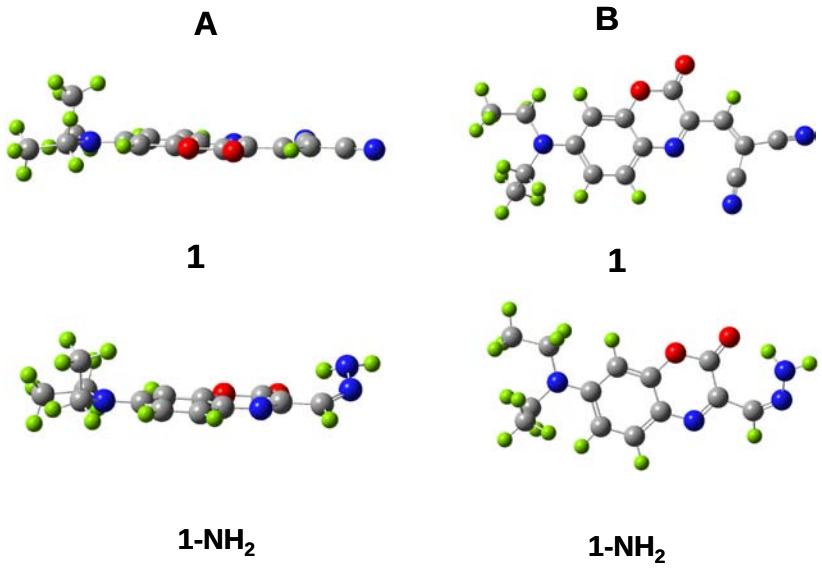


Figure S17. Optimized ground-state geometries of the reactant **1** and the corresponding product **1-NH$_2$**. A) The side-view; B) The top-view

Table S2. Experimental and theoretical spectra of compound **1** and the responding compound **1-NH$_2$**.

Compound	Exptl λ(nm)		Calcd λ(nm)	
	Abs.	Fl.	Abs.	Fl.
reactant	598	639	516	614
product	480	564	471	597

Table S3. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI coefficients of the low-lying excited states of the reactant **1** and the corresponding product **1-NH$_2$**. The data were calculated by TDDFT//B3P86/TZVP based on the optimized ground state geometries.

compoud	Electronic Transition	TDDFT //B3P86/TZVP			
		Excitation Energy[a]	f[b]	Composition[c]	CI[d]
reactant	$S_0 \rightarrow S_1$	2.40 eV 516 nm	1.1798	H→L	0.7103
	$S_0 \rightarrow S_2$	3.19 eV 388 nm	0.0009	H-3→L	0.6170
	$S_0 \rightarrow S_3$	3.28 eV 378 nm	0.0031	H-1→L	0.6799
	$S_0 \rightarrow S_4$	3.79 eV 327 nm	0.0333	H-2→L H→L+1	0.4445 0.4793
	$S_0 \rightarrow S_5$	3.98 eV 311 nm	0.1173	H-1→L+1	0.5048
	$S_0 \rightarrow S_6$	4.62 eV 268 nm	0.0155	H→L+2	0.6883
product	$S_0 \rightarrow S_1$	2.63 eV 471 nm	0.7554	H→L	0.7020
	$S_0 \rightarrow S_2$	3.48 eV 356 nm	0.0282	H-1→L	0.6739
	$S_0 \rightarrow S_3$	3.72 eV 333 nm	0.0013	H-3→L	0.5965
	$S_0 \rightarrow S_4$	3.86 eV 321 nm	0.0155	H-2→L	0.6501
	$S_0 \rightarrow S_5$	4.15 eV 298 nm	0.0058	H-4→L	0.6276
	$S_0 \rightarrow S_6$	4.19 eV 295 nm	0.2091	H→L+1	0.6625

[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength . [c] H stands for HOMO and L stands for LUMO. [d]The CI coefficients are in absolute values.

XYZ coordinates (angstrom)

Note: upper case letters before the atomic coordinates indicate the atomic symbol of the atoms in volved in the calculations.

Reactant in the S_0 state

C	2.55695200	-0.21940900	-0.17591900
C	1.87102200	-1.47991900	-0.23914200
C	0.51543000	-1.54761500	-0.21044500

C	-0.27921600	-0.37861800	-0.09847300
C	0.41001700	0.86022400	-0.01724300
C	1.76954000	0.95642100	-0.04523300
N	-1.60524400	-0.44208700	-0.06699500
C	-2.31287400	0.67084700	0.04766900
C	-1.68464500	1.98957700	0.15385900
O	-0.31495000	2.00409100	0.10957200
O	-2.24959300	3.05002500	0.27407700
C	-3.73172300	0.64058300	0.07403900
C	-4.55545900	-0.44764400	-0.00523900
C	-5.96201300	-0.25813100	0.03503600
N	-7.10712300	-0.10994800	0.06689700
C	-4.13777700	-1.79853100	-0.12873500
N	-3.89310900	-2.92277700	-0.22898100
N	3.89584900	-0.14687200	-0.23735500
C	4.72156600	-1.35443800	-0.22892100
C	4.54884400	1.16817600	-0.10865900
C	6.03292700	1.18855900	-0.40514000
C	4.96562000	-1.89852300	1.17032200
H	2.43192000	-2.39878000	-0.31112900
H	0.00892600	-2.50331300	-0.26409300
H	2.21410500	1.93567500	0.04043600
H	-4.21770100	1.60457700	0.16740800
H	5.66605300	-1.10818800	-0.70534100
H	4.25513000	-2.10411000	-0.86533200
H	4.37016700	1.54691900	0.90311900
H	4.05235800	1.84995800	-0.80105800
H	6.37021100	2.22214500	-0.31400600
H	6.25133900	0.85692300	-1.42127100
H	6.61290200	0.58963600	0.29728900
H	5.59946900	-2.78515100	1.11204300
H	4.02967200	-2.17825200	1.65703300
H	5.46991100	-1.15852700	1.79409200

Reactant in the S_1 state

C	2.55950000	-0.22688400	-0.18465600
C	1.88242400	-1.48287900	-0.24782500
C	0.51707700	-1.55057500	-0.20905500
C	-0.27992700	-0.39092400	-0.10174600
C	0.40204700	0.84045900	-0.02705500
C	1.76864600	0.94281300	-0.06258300
N	-1.63434200	-0.47892000	-0.06928800
C	-2.31924100	0.65843900	0.05039400
C	-1.66950700	1.98313000	0.15623600

O	-0.30986300	2.00276400	0.10183500
O	-2.24098900	3.03881200	0.28209600
C	-3.72681400	0.67197000	0.08066200
C	-4.56064500	-0.43712700	-0.00194100
C	-5.96014900	-0.24575000	0.03530300
N	-7.10692100	-0.08571700	0.06563900
C	-4.15785200	-1.78567200	-0.12329900
N	-3.94371200	-2.92022700	-0.22258500
N	3.91340700	-0.14479500	-0.23926900
C	4.73766400	-1.34445700	-0.22990000
C	4.55505200	1.16897400	-0.12220500
C	6.04571200	1.19753600	-0.37781100
C	4.97148400	-1.89518000	1.17477200
H	2.44011400	-2.40370700	-0.32513000
H	0.01585300	-2.50902700	-0.26041500
H	2.20603000	1.92642700	0.01672300
H	-4.20496800	1.63742900	0.17551600
H	5.68847500	-1.09472100	-0.69223300
H	4.27516700	-2.09698400	-0.86681700
H	4.34569400	1.56438300	0.88004500
H	4.06562300	1.83984900	-0.83274800
H	6.37111100	2.23473500	-0.28627300
H	6.29370900	0.85949600	-1.38494300
H	6.60986800	0.60936900	0.34604200
H	5.60512500	-2.78130100	1.10862400
H	4.03031900	-2.17614900	1.64958600
H	5.47284300	-1.15803100	1.80334200

Product in the S_0 state

C	-2.02556700	-0.22462300	-0.22794400
C	-1.63631200	-1.59241200	-0.36531600
C	-0.32167100	-1.96022900	-0.41171100
C	0.70792800	-1.00405500	-0.32060300
C	0.31465400	0.33557900	-0.17536900
C	-0.99616600	0.73869700	-0.12569900
N	2.00944300	-1.35904900	-0.32539600
C	2.94652100	-0.45036600	-0.19284100
C	2.61083100	0.97229500	-0.14527700
O	1.28745400	1.28803400	-0.09564400
N	-3.33225900	0.14113800	-0.20727500
C	-4.38418500	-0.86351500	-0.08828200
C	-3.66310500	1.55228200	0.02564000
C	-5.12135100	1.91328700	-0.16790500
C	-4.58951100	-1.36388100	1.33403600

O	3.39266400	1.90350100	-0.17461400
C	4.31826000	-0.96654200	-0.21467900
N	5.43723700	-0.54686100	0.26209100
N	5.55930000	0.59003400	0.96581200
H	-2.38776500	-2.36464700	-0.42617300
H	-0.04651200	-3.00319600	-0.51322900
H	-1.20166900	1.79027400	0.00238300
H	-4.16042900	-1.69095200	-0.76064700
H	-5.30401600	-0.42344000	-0.46437400
H	-3.07231500	2.14954900	-0.67195800
H	-3.34624900	1.83736400	1.03647000
H	-5.21847800	2.99165200	-0.03237000
H	-5.77647000	1.43033400	0.55788800
H	-5.47002000	1.67141800	-1.17348000
H	-5.39194300	-2.10403100	1.35507900
H	-4.86574900	-0.54353900	1.99933300
H	-3.68393600	-1.83193000	1.72448200
H	4.37108500	-1.95513500	-0.66171600
H	6.52466900	0.87361900	1.03779300
H	4.91084600	1.34444100	0.74166300

Product in the S_1 state

C	-2.02640000	-0.23547700	-0.21824000
C	-1.65182000	-1.60405900	-0.34320700
C	-0.33547600	-1.97711200	-0.37099100
C	0.71837700	-1.03455000	-0.28963200
C	0.32822400	0.31733500	-0.15561400
C	-0.98114700	0.71939400	-0.12259900
N	2.00539600	-1.42264800	-0.30767500
C	2.95022300	-0.44260500	-0.15649300
C	2.62791900	0.94502900	-0.09610500
O	1.28546700	1.29211400	-0.07259500
N	-3.32895800	0.15347100	-0.20021000
C	-4.39655300	-0.83549200	-0.13192200
C	-3.64735700	1.57015200	0.01106400
C	-5.10271500	1.94472500	-0.17333200
C	-4.64939700	-1.35362700	1.27885700
O	3.39595900	1.91598400	-0.08822500
C	4.28214800	-0.98989900	-0.14224800
N	5.45780400	-0.55257200	0.24847200
N	5.60333600	0.65599700	0.73275000
H	-2.40628700	-2.37371800	-0.40367100
H	-0.06954400	-3.02369200	-0.46200100
H	-1.18237900	1.77404900	-0.00958900

H	-4.15880500	-1.65727100	-0.80656700
H	-5.29911500	-0.37640900	-0.52621900
H	-3.05187500	2.15288900	-0.69523800
H	-3.31950100	1.86019100	1.01728800
H	-5.18521300	3.02369000	-0.03354100
H	-5.75776100	1.46729700	0.55591100
H	-5.45991300	1.71163400	-1.17771300
H	-5.45701900	-2.08788300	1.25826800
H	-4.94243400	-0.54105700	1.94586800
H	-3.75888400	-1.83328300	1.68827000
H	4.30650000	-2.02696500	-0.46023500
H	6.54946900	0.90775100	0.97645500
H	4.88215400	1.37537700	0.56867900

13. NMR and MS data for compound 1

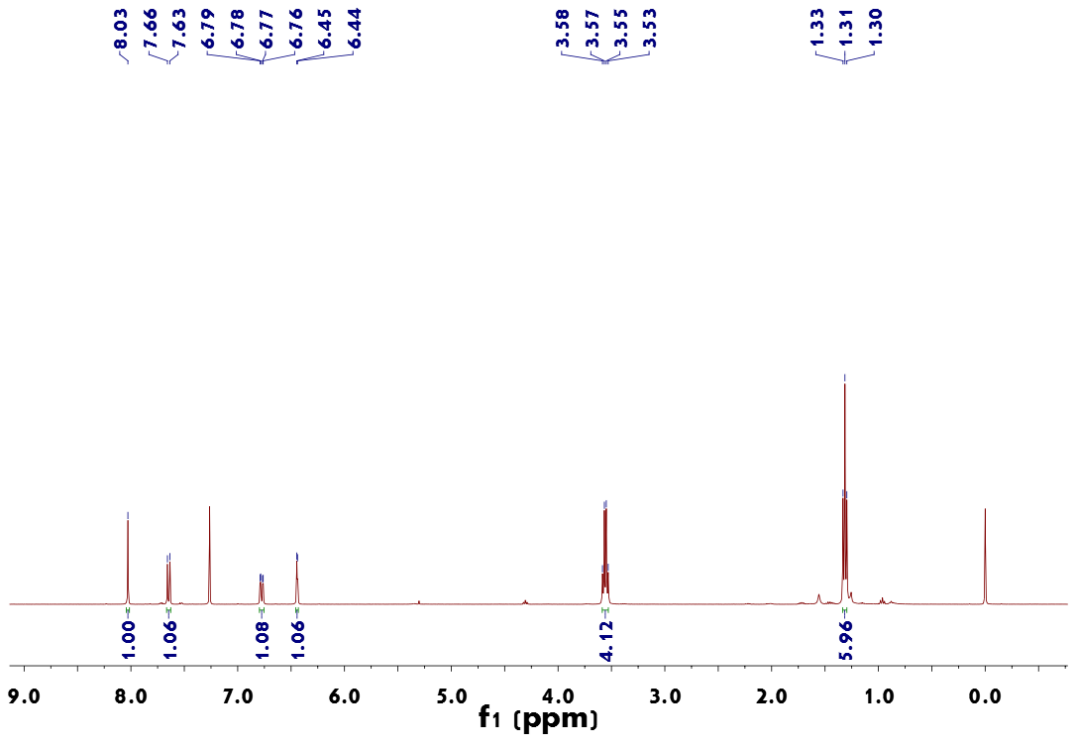


Figure S18. ^{1}H NMR spectrum of compound **1** in $CDCl_3$

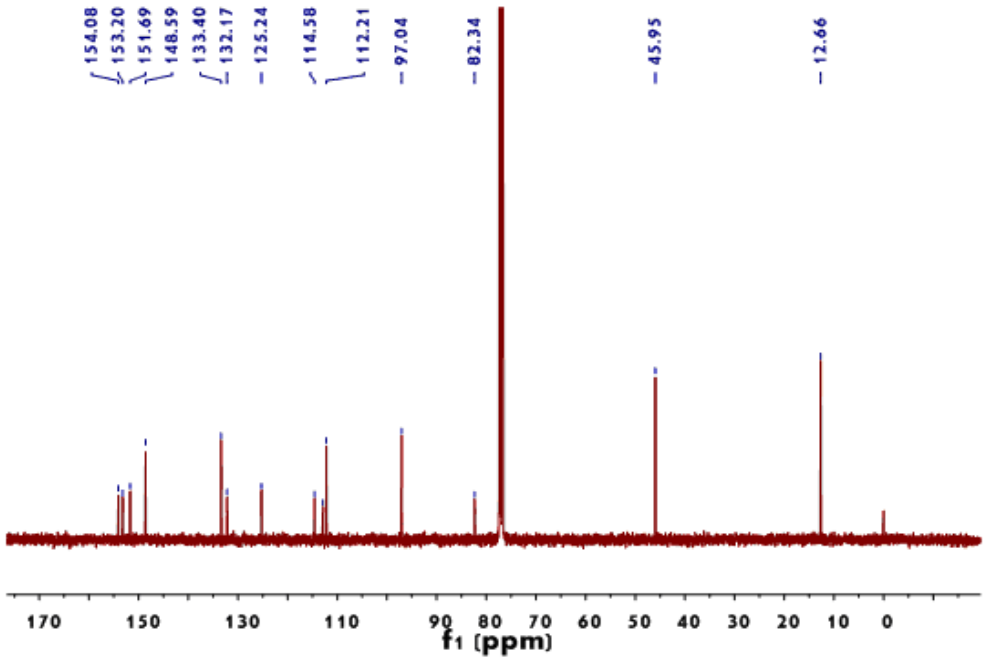
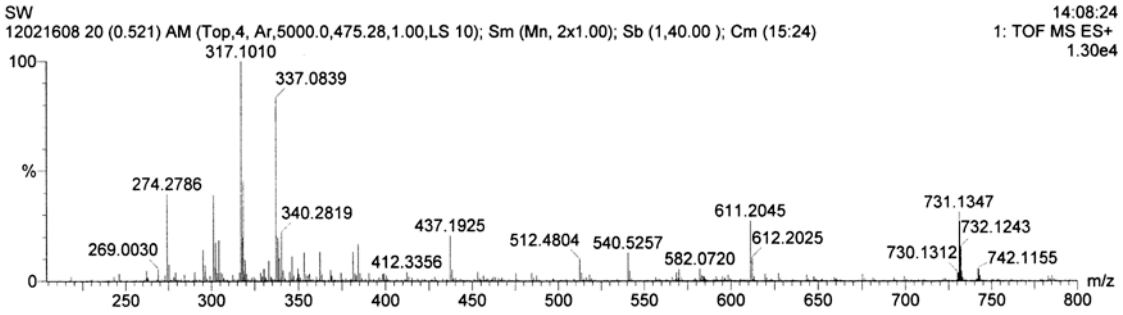


Figure S19. ^{13}C NMR spectrum of compound **1** in $CDCl_3$

Single Mass Analysis (displaying only valid results)
Tolerance = 5.0 PPM / DBE: min = -200.0, max = 200.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
13 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-150 H: 0-150 N: 4-4 O: 2-2 Na: 1-1



Minimum:				-200.0		
Maximum:		5.0	5.0	200.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
317.1010	317.1014	-0.4	-1.3	11.5	15.5	C16 H14 N4 O2 Na

Figure S20. TOF-MS spectrum of compound **1**