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A single rhodamine spirolactam probe for localization and pH monitoring of mitochondrion/lysosome in living cells

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1. General methods and reagents

The reagents such as $ClCH_2CH_2Cl$, $POCl_3$, THF, acetonitrile and triethylamine were purchased from commercial suppliers and used without further purification. 2-nitroaniline and $LiAlH_4$ were purchased from Ouhe Chem. Column chromatography was performed with silica gel (300-400 mesh). RPMI 1640 culture medium with L-glutamine, rhodamine 123 and LysoSensor™ Green DND-189 were purchased from ThermoFisher Scientific (Invitrogen, USA), FBS (fetal calf serum) was purchased from ThermoFisher Scientific (Invitrogen, USA).

^1H NMR and ^{13}C NMR were measured on Varian MERCURY 500 MHz spectrometer in $CDCl_3$ and DMSO-d6 with TMS as internal reference. Mass spectra were measured on a HP 1100 LC-MSD, Gas chromatography/TOF Mass spectrometers and the UPLC/Q-TOF Mass spectrometers. Fluorescence spectra were measured on Spectrofluorophotometer (Cary Eclipse). Absorbance spectra were recorded on a UV-vis Spectrophotometer (TU-1901). An inverted confocal fluorescent microscopy (Olympus FV1000, IX81, Olympus, Japan) equipped with an objective lens (x100 oil, 1.4 Numerical Aperture (NA), Scan mode XY) was used in the imaging of living cells.

2. Solvent effect

During testing of solvent effect, 300 uL Rh-BMDZ stock solution was added into 10 mL glass bottle containing different solvents, such as dichloromethane (DCM), tetrahydrofuran (THF), methanol, ethanol, acetonitrile, acetone.

3. pH titration

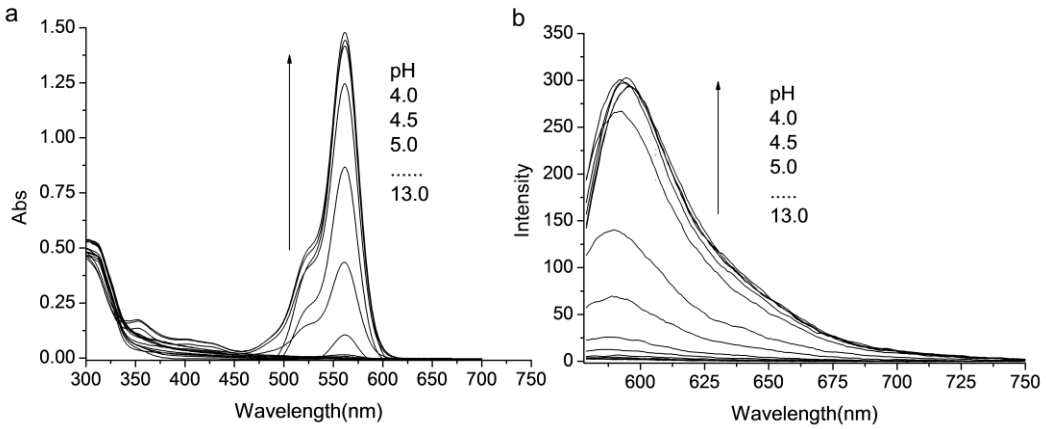


Fig. S1 Absorption (a) and emission (b) spectra of Rh-BMDZ in aqueous solution (CH_3CN:H_2O 1:1,v:v) vs various pH values

4. Spectral changes of Rh-ClO vs different metal ions

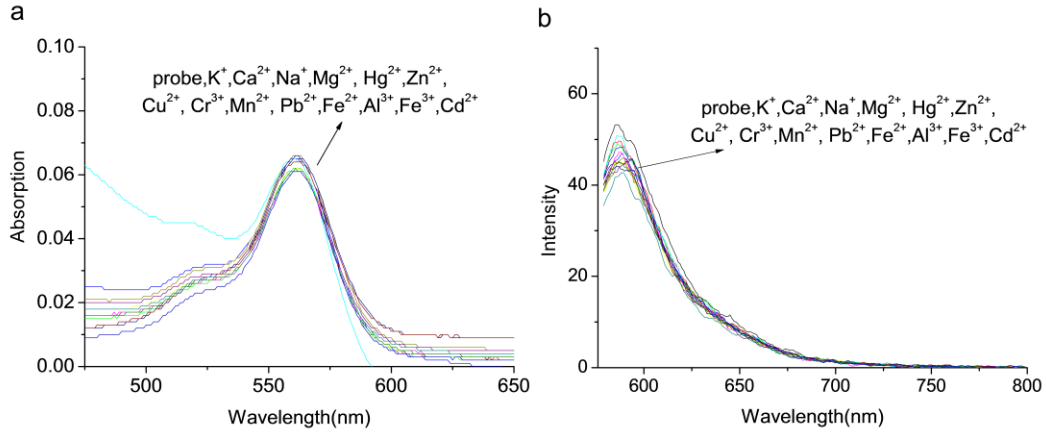


Fig. S2 Absorption (a) and emission (b) spectra of Rh-BMDZ in CH_3CN/tris-HCl buffer (1:1, v:v, pH 7.4) in the presence of metal ions such as K^+, Ca^{2+}, Na^+, Mg^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Cd^{2+}, Hg^{2+}, Mn^{2+} and Al^{3+}.

5. Spectral changes of Rh-BMDZ in various solvents

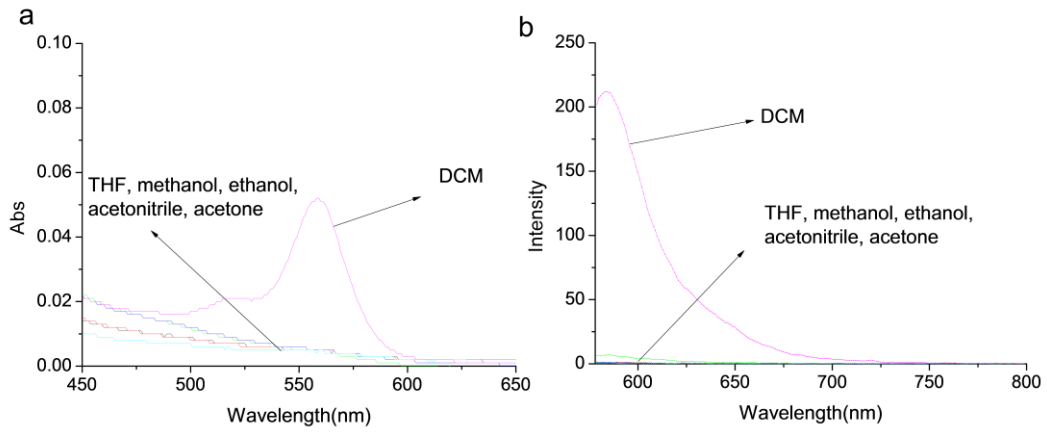


Fig. S3 Absorption (a) and emission (b) spectra of Rh-BMDZ in DCM, THF, methanol, ethanol, acetonitrile and acetone.

6. Fluorescence image

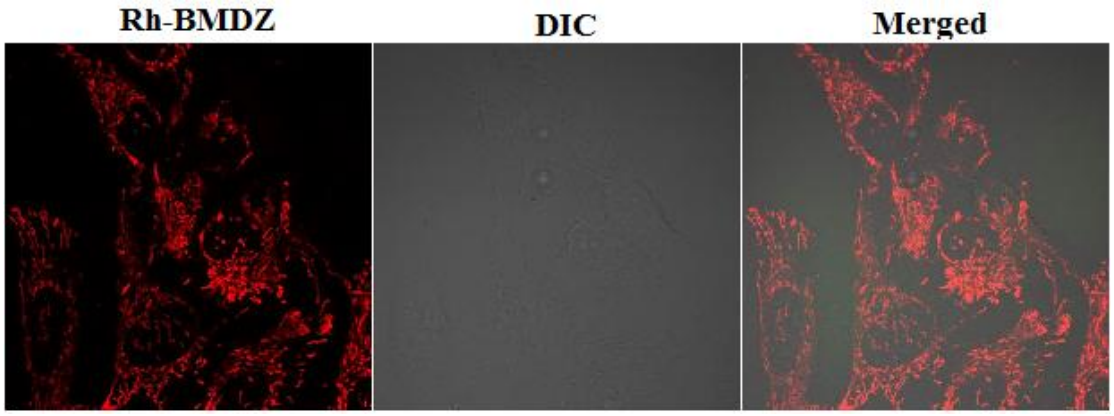


Fig. S4 MCF-7 stained with Rh-BMDZ (2 uM) for 5 min. Rh-BMDZ: λex 559 nm, λem 565-665 nm, DIC: differential interference contrast, Merged image: Overlay of Rh-BMDZ and DIC.

7. Intensity correlation analysis

Table S1 ICA data of Fig. 3a(top), 3a(bottom) and Fig.3c (top)

Ch1 and Ch2	Rr [a]	R [b]	Ch1:Ch2 [c]	M_1 [d]	M_2 [d]	N+ve [e]	Ntotal [f]	ICQ [g]
Fig.3a (top)	0.84	0.85	1	0.263	0.662	174404	194336	0.40
Fig.3a (bottom)	0.52	0.51	1	0.032	0.218	156751	191449	0.32
Fig.3c (top)	0.85	0.87	1	0.995	0.989	240819	261976	0.42

a Pearson's correlation coefficient. b Mander's Overlap coefficient, c This value represents the red: green pixel ratio. d These split coefficients are Mander's Colocalization coefficients for Ch 1 (M1) and Ch 2 (M2). e N+ve represents the number of pixel pairs that have a positive PDM value. f Ntotal is the number of pixels pairs in the images that where at least one of the pixel pairs is above zero. g ICQ is the Intensity Correlation Quotient. Dependent staining: 0<ICQ<+0.5.

8. Cytotoxicity assay of Rh-BMDZ

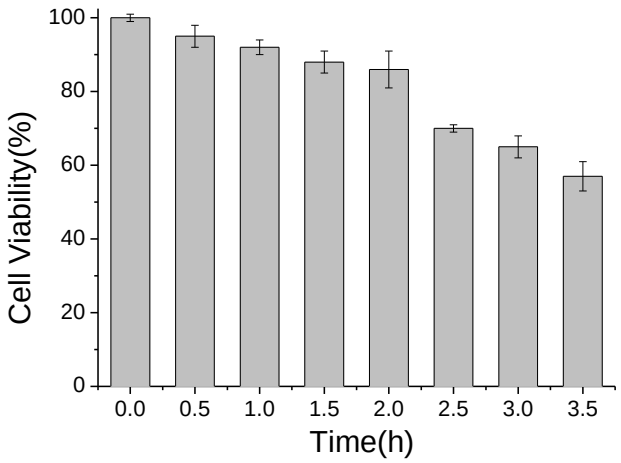
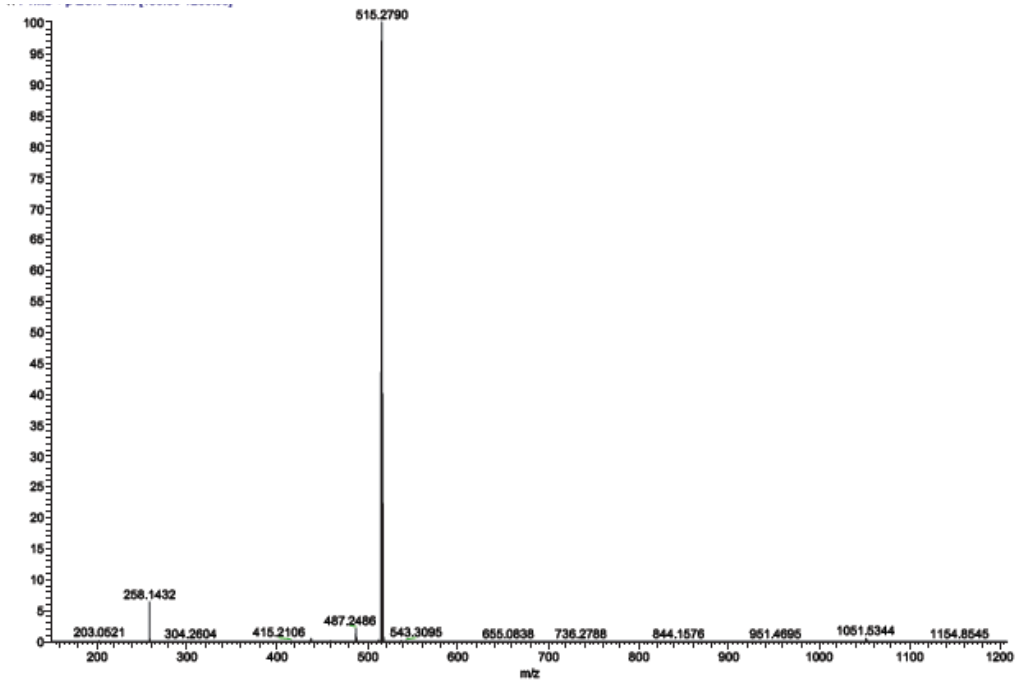


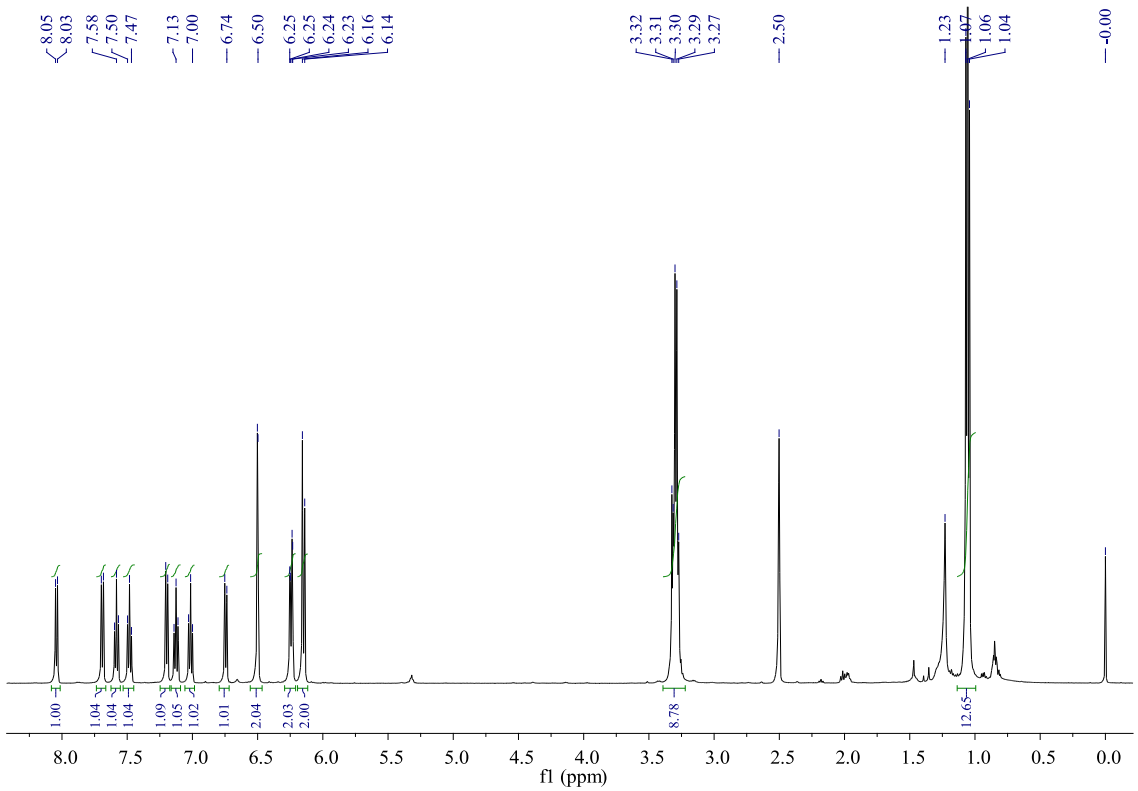
Fig. S5 The cytotoxicity effect of Rh-BMDZ on MCF-7. MCF-7 cells were incubated with Rh-BMDZ (2 uM, containing 2% DMSO) during different interval at 37 ˚C. Results were expressed as mean ± standard deviation (SD) of three independent experiments.

9. HRMS and H/C-NMR

① HRMS



② ^{1}H-NMR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7.58
7.50
7.47
7.13
7.00
6.74
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6.25
6.25
6.24
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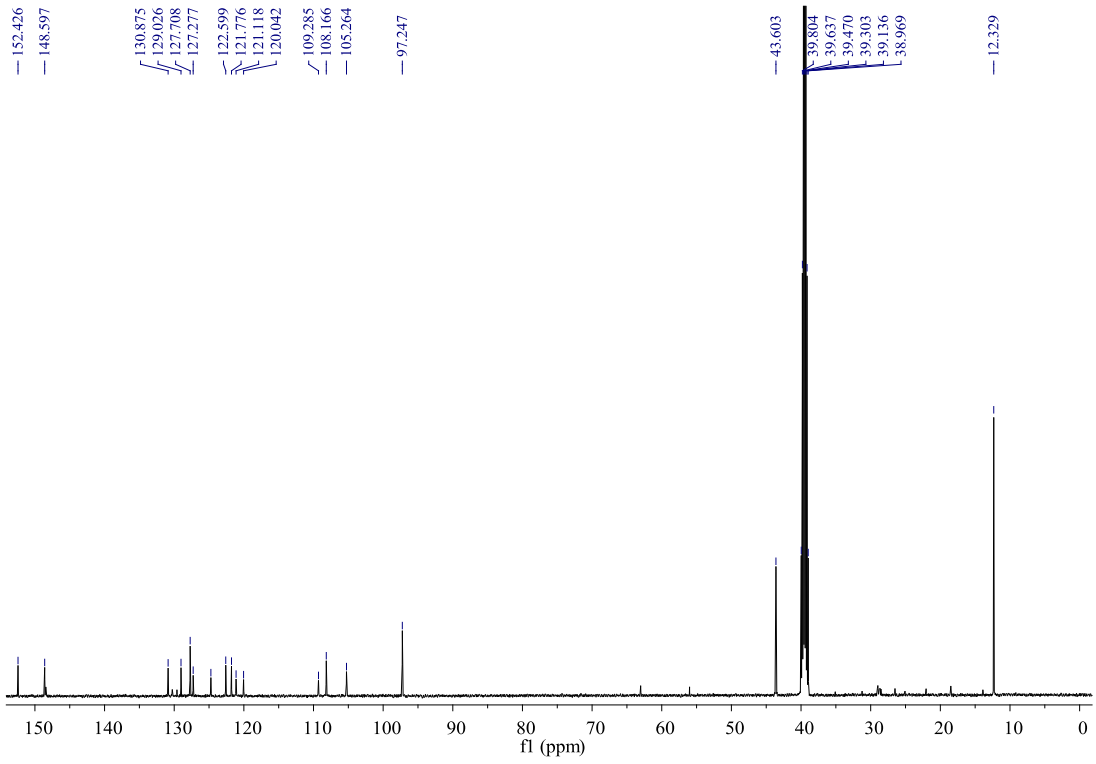
6.16
6.14
3.32
3.31
3.30
3.29
3.27
2.50
1.23
1.07
1.06
1.04
-0.00
1.00
1.04
1.04
1.04
1.09
1.05
1.02
1.01
2.04
2.03
2.00
8.78
12.65
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

③ ^{13}C-NMR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129.026
127.708
127.277
122.599
121.776
121.118
120.042
109.285
108.166
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97.247
43.603
39.804
39.637
39.470
39.303
39.136
38.969
12.329
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)