Synthesis of a Golgi-targeting fluorescent probe for the selective detection of chloride anions

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Supplementary Information

Experimental sections

Generals. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance AV 500 NMR spectrometer. HRMS spectra were measured on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer. Absorption and fluorescence spectra were measured on a U-3900 ultraviolet-visible spectrophotometer and Perkin Elmer FL8500 fluorescence spectrophotometer, respectively. Staining experiments with organelle-targeting dyes were carried on a Leica TCS SP8 microscope.

Golgi-Tracker Red, Mito-Tracker Red, Lyso-Tracker Red and ER-Tracker Red dyes were purchased from Shanghai Biyuntian Biotechnology Co., LTD. All the other reagents were analytically pure.

1. Chemistry

*Synthesis of compound **2**.* To a solution of *p*-aminobenzenesulfonamide (1.72 g, 10 mmol) in acetone (10 mL) was added dropwise bromoacetyl bromide (0.82 mL, 9.5 mmol) at 0 °C. The resulting solution was heated at 55 °C for 10 min. Then the reaction was quenched with iced water and the reaction mixture was stirred for further 8 h. The formed solid was collected through reduced pressure, washed with iced water and recrystallized from ethanol to give compound **2** (980 mg, 35%) having ^{1}H NMR (500

MHz, DMSO-d_6) δ 10.72 (s, 1H), 7.79 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 8.9 Hz, 2H), 7.29 (s, 2H), 4.07 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.0, 141.9, 138.9, 127.0, 119.6, 62.3 and HR-ESI-MS for $C_8H_9N_2O_3S$ ($[M+H]^+$) Calcd: 292.9592, Found: 292.9590.

*Synthesis of compound **1***. A solution of 6-methoxyquinoline (255 mg, 1.6 mmol) and compound **2** (235 mg, 0.8 mmol) in DMF (6 mL) was heated at 100 ℃ for 8 h. The reaction mixture was diluted with water and washed with dichloromethane. The aqueous layer was frozen-dried to afford compound **1** (300 mg, 83%) having ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.39 (d, J = 4.3 Hz, 1H), 9.23 (d, J = 8.1 Hz, 1H), 8.41 (d, J = 9.6 Hz, 1H), 8.22 (dd, J = 8.1, 4.3 Hz, 1H), 7.95 (d, J = 2.9 Hz, 1H), 7.90 (dd, J = 9.6, 2.9 Hz, 1H), 7.79 (d, J = 8.9 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 7.31 (s, 2H), 6.08 (s, 2H), 4.01 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.0, 159.7, 148.8, 147.0, 141.4, 139.7, 134.8, 131.9, 128.6, 127.4, 122.8, 121.0, 119.5, 108.6, 59.9, 56.9 and HR-ESI-MS calcd for $C_{18}H_{18}N_3O_4S^+$ ($[M–Br]^+$) 372.1012, found 372.1010.

2. Cell viability of compound **1**

Cell viability was assessed by using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. [1] Human cervical cancer cells were cultured in DMEM (Cytiva) supplemented with 10% fetal bovine serum (FBS), 50 units/mL penicillin and 50 units/mL streptomycin. The cells were maintained at 37 °C under a humidified atmosphere containing 5% CO_2. HeLa cells at a density of 8×10^3 cells/well (per 100 μL) were plated in 96-well plates for 24 h and incubated with compound **1** of varying concentrations in culture media for 12 h. Then, 10 μL of MTT (5 mg/mL) was added to culture media in each well and the mixture was then incubated for 4 h. After removing the culture media containing MTT, 100 μL of DMSO was added. The absorbance at 570 nm was measured using a Synergy Neo2 multimode microplate reader. All the experiments were repeated independently thrice. The cell viability was calculated using the following formular.

$$\text{Cell viability\%} = (OD_{Treated} - OD_{Blank})/(OD_{Control} - OD_{Blank}) \times 100\%.$$

3. Golgi-targeting properties [2]

HeLa cells were incubated in DMEM supplemented with 10% fetal bovine serum (FBS), 50 units/mL penicillin and 50 units/mL streptomycin for 24 h and then treated with Golgi-Tracker Red (150 μg/mL), Mito-Tracker Red (1 μM), Lyso-Tracker Red (1 μM) and ER-Tracker Red (1 μM) for 30 min, respectively. The cells were washed three times with PBS. Then chlorine-free HBSS solution containing compound **1** (2 mM) was added. After incubation for 2.5 h, the cells were washed three times with chloride anion-free HBSS solution and imaged with a laser confocal microscope. All the experiments were repeated independently thrice. Red channel: Golgi-Tracker Red with excitation at 552 nm and emission at 590−690 nm, Mito-Tracker Red with excitation at 552 nm and emission at 580–680 nm, ER-Tracker Red with excitation at 552 nm and emission at 600−700 nm, and Lyso-Tracker Red with excitation at 552 nm and emission at 580–650 nm. Blue channel: compound **1** with excitation at 405 nm and emission at 400–550 nm.

The Pearson co-localization coefficient is calculated using an Image-Pro Plus software as follows:

(1) Open the image and navigate to the "Measure" tab. Then, select "Co-localization" and choose the desired colors (blue and red) in the pop-up dialog box. Finally, click "Calculate Correlation" to obtain the Pearson coefficient. A dialog box will appear, allowing one to save the data. See Fig. S9-S11 and Table S1 for details.

(2) After obtaining the Pearson coefficient, click "Co-localization" again and proceed to the next step. This will bring up a scatter plot. Save the scatter plot in TIFF format for further analysis.

4. Detection of chloride anions in Golgi apparatus

HeLa cells were incubated at a density of 5×10^4 cells/well for 24 h. After the supernatant was removed, the cells were washed with chlorine-free HBSS solution (1 mL×3). Then, 1 mL of chlorine-free HBSS solution containing 10% FBS [3] was added and the cells were cultured in an incubator at 37 °C for 6 h. After the supernatant was removed, the cells were washed with chlorine-free HBSS solution (1 mL×3). Then,

HBSS solution containing compound **1** (2 mM) and chloride anions of varying concentrations was added, and the cells were cultured at 37 °C for 2.5 h. After the supernatant was removed, the cells were washed with HBSS solution containing the corresponding concentration of chloride anions (1 mL×3). Then 1 mL of HBSS solution containing the corresponding concentration of chloride anions was added. Finally, the cells were imaged with a laser confocal microscope and the appropriate area was selected for photographing. All the experiments were repeated independently thrice.

The blue fluorescence was calculated quantitatively using an Image*J* software. For a single channel (monochrome) fluorescent image, the gray value of each pixel represents the magnitude of fluorescence intensity at that point and is calculated according to *Mean gray value = Integrated Density / Area*. This calculation is performed to determine the average intensity of the fluorescence signal within a defined region. The details of the operation are described below.

(1) Open the image and split the channels using the "Image-Color-Split Channels" option, ensuring that only the relevant channel is selected for further analysis.

(2) Set the analysis parameters using the "Analyze-Set Measurements" and select the desired parameters for measurement. This typically includes area, mean gray value, integrated density and limit to the threshold.

(3) To obtain the average gray value, apply an appropriate thresholding algorithm to the image. It is essential to use the same thresholding algorithm consistently for all images in the analysis. This can be achieved by accessing "Image-Adjust-Threshold(default)-Analyze-Measure".

5. Statistics section

All the statistical analyses were conducted using GraphPad Prism software and Origin software. Data are presented as mean ± s.d. from three independent experiments. The statistical significance of differences in measured variables between two groups was assessed using an unpaired Student's t-test and between multiple groups and conditions using one-way and two-way ANOVAs. A value of $p < 0.05$ is considered statistically significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

References

1. S. H. Park, J. Y. Hyun and I. Shin, A lysosomal chloride ion-selective fluorescent probe for biological applications. *Chem. Sci.*, **2019**, *10 (1)*, 56–66.
2. H. Wang, Z. He, Y. Yang, J. Zhang, W. Zhang, W. Zhang, P. Li and B. Tang, Ratiometric fluorescence imaging of Golgi H_2O_2 reveals a correlation between Golgi oxidative stress and hypertension. *Chem. Sci.*, **2019**, *10 (47)*, 10876–10880.
3. X. H. Yu, C. C. Peng, X. X. Sun and W.-H. Chen, Synthesis, anionophoric activity and apoptosis-inducing bioactivity of benzimidazolyl-based transmembrane anion transporters. *Eur. J. Med. Chem*., **2018**, *152*, 115–125.

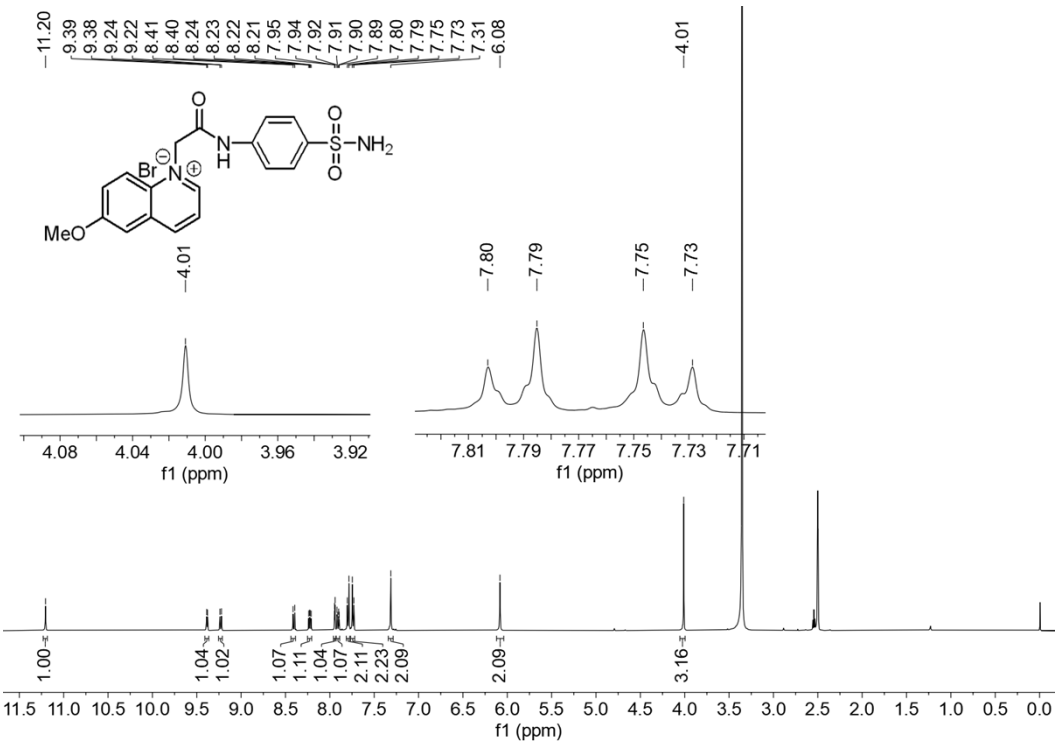

Fig. S1. ^{1}H NMR (500 MHz, DMSO-d_6) of compound **1**.

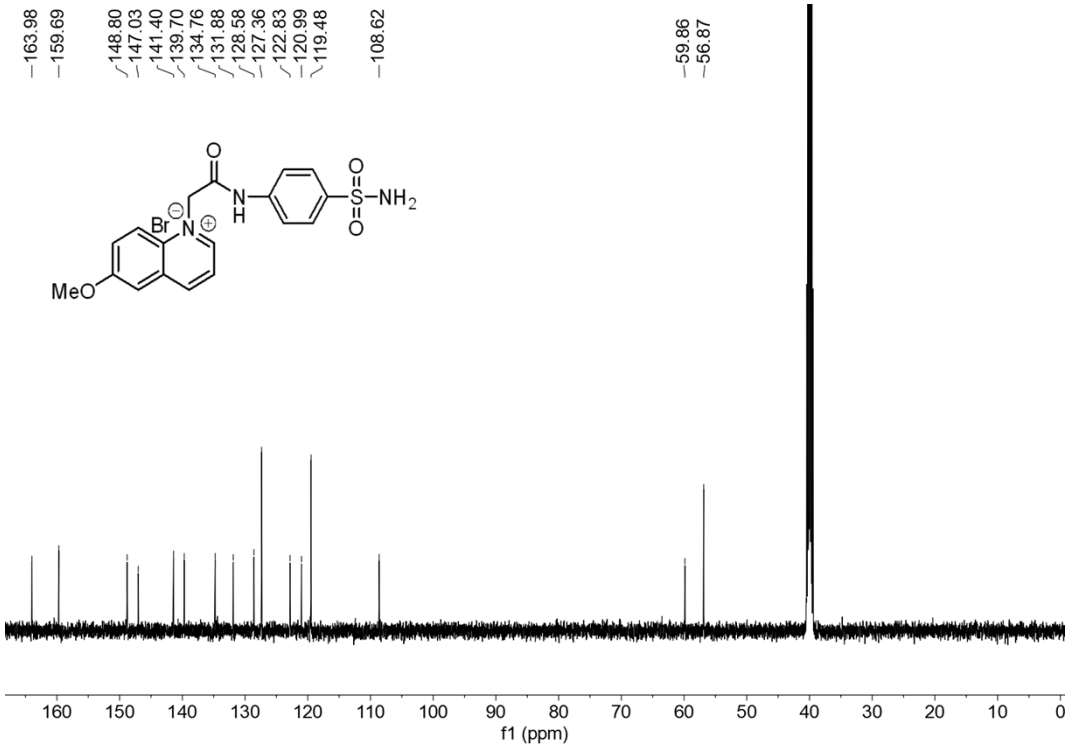

Fig. S2. ^{13}C NMR (125 MHz, DMSO-d_6) of compound **1**.

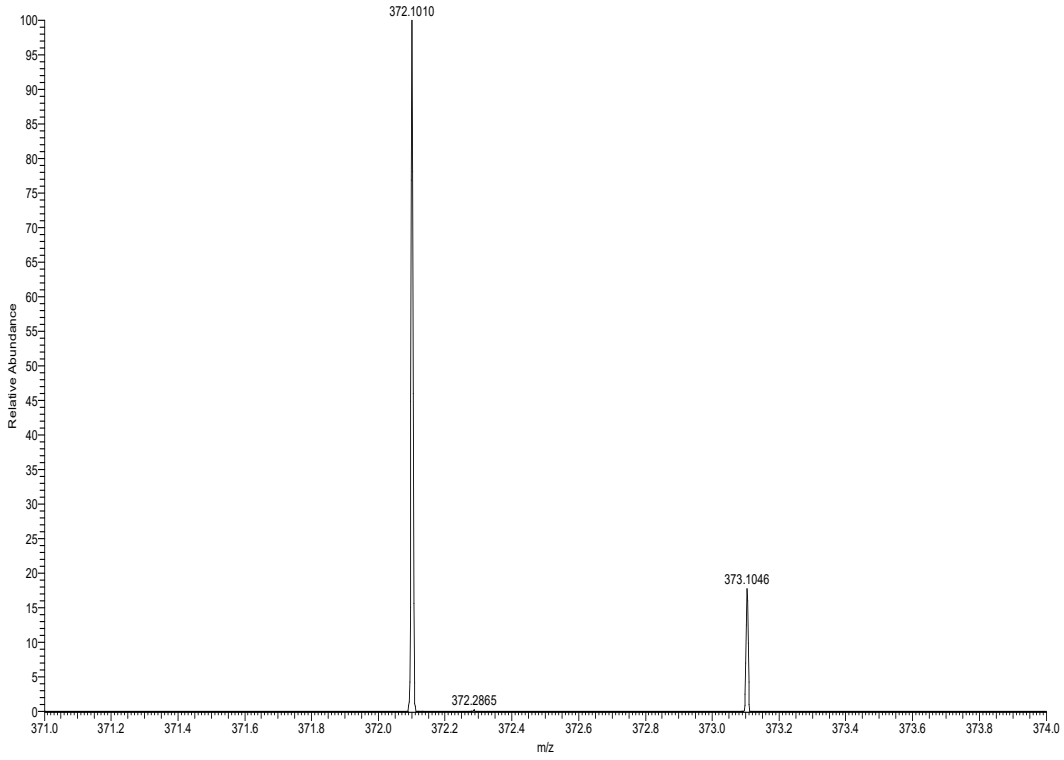

Fig. S3. HR-ESI-MS of compound **1**.

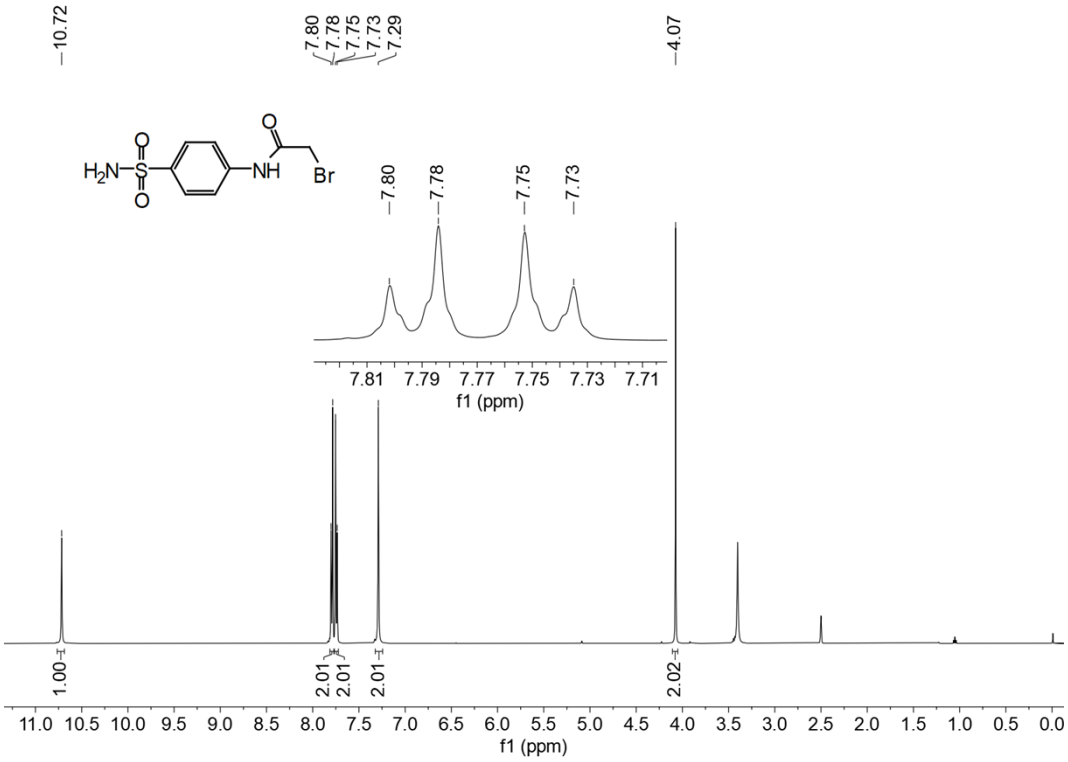

Fig. S4. ^{1}H NMR (500 MHz, DMSO-d_6) of compound **2**.

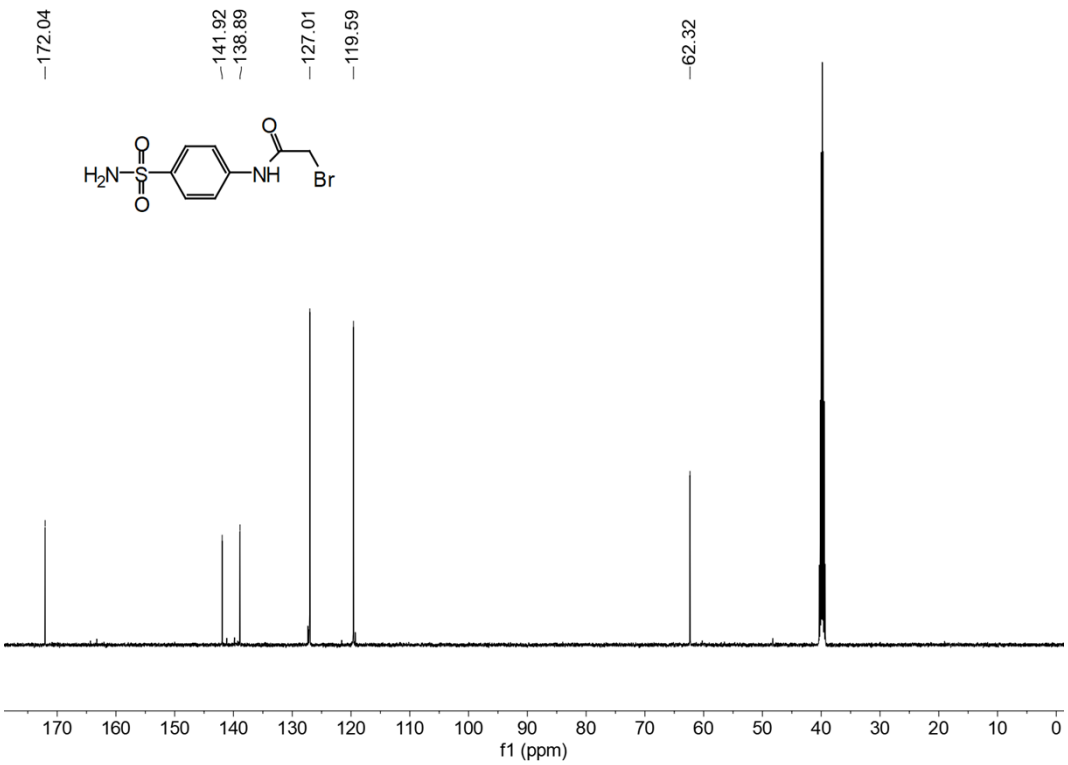

Fig. S5. ^{13}C NMR (125 MHz, DMSO-d_6) of compound **2**.

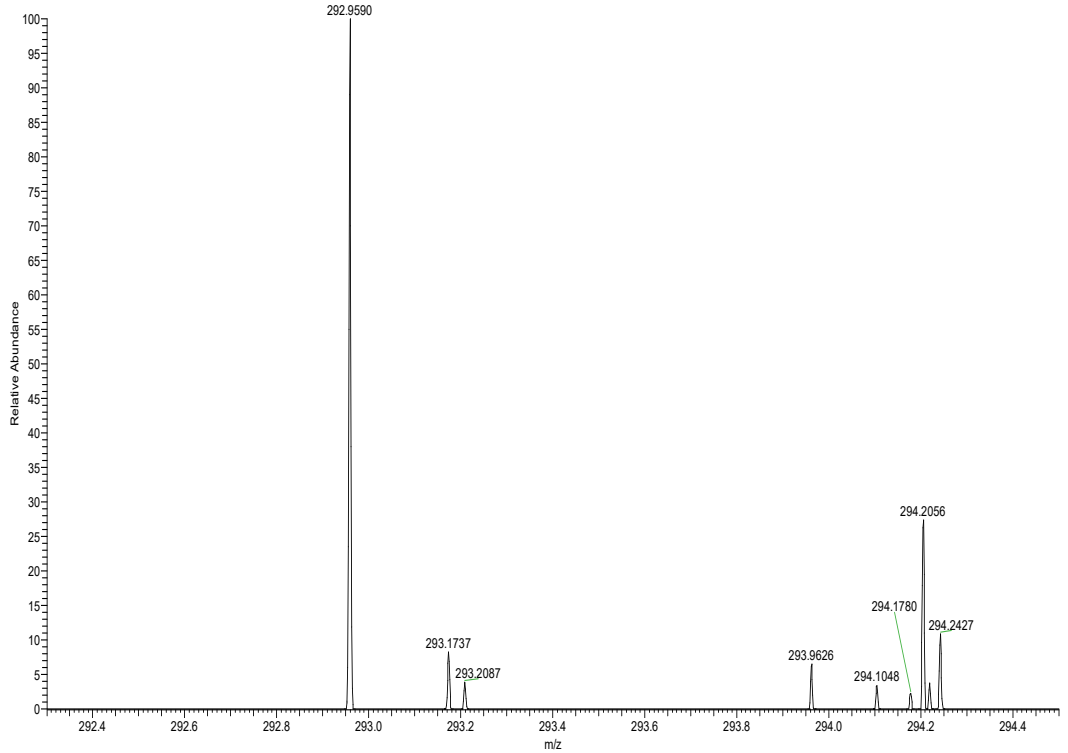

Fig. S6. HR-ESI-MS of compound **2**.

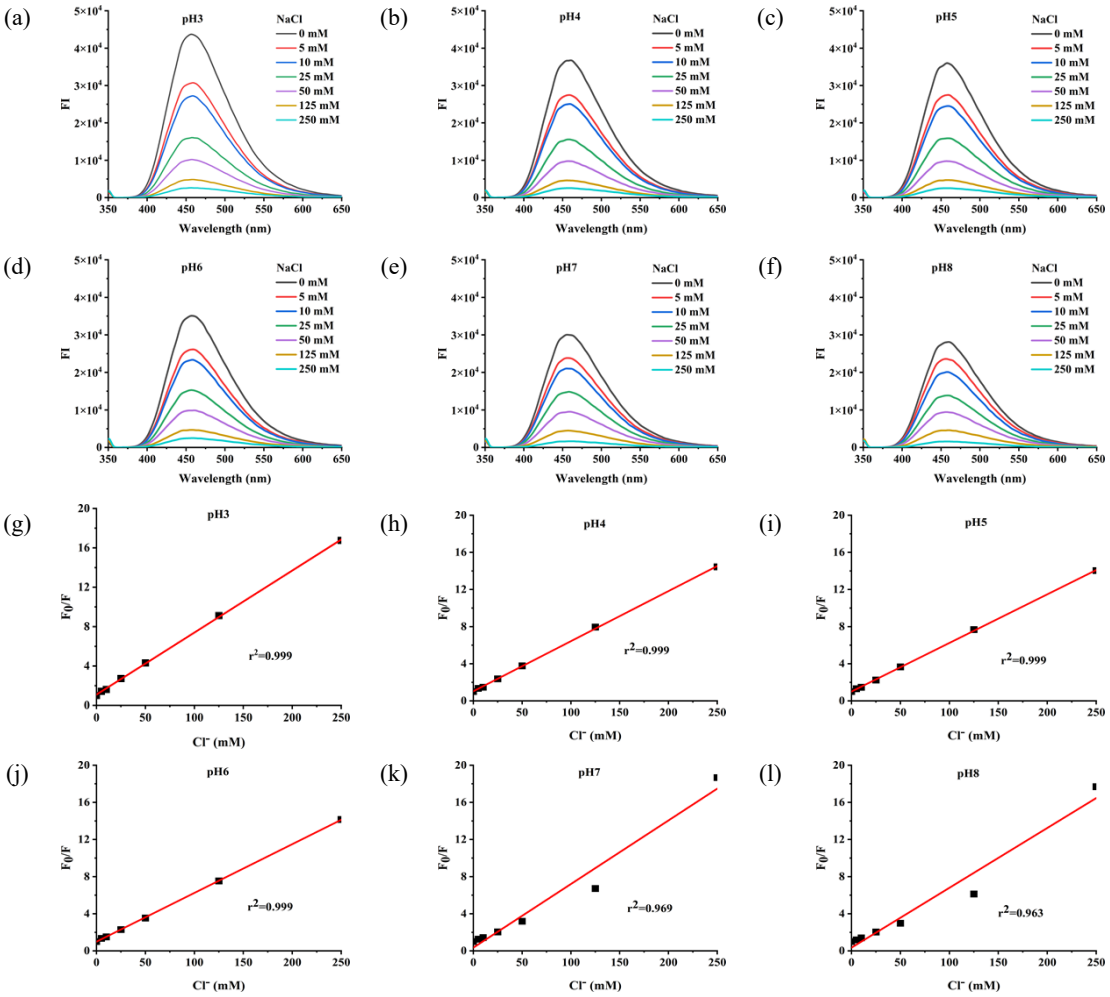

Fig. S7. (a-f) Typical fluorescence spectra of compound **1** (100 μM) at different pH and varying concentrations of chloride anions. (g-l) Typical Stern-Volmer plots of the relative fluorescent intensity (F_0/F) of compound **1** (100 μM) against the concentration of chloride anions at each pH. The used media was 100 mM phosphate buffer. $\lambda_{ex}/\lambda_{em}$ = 350/453 nm.

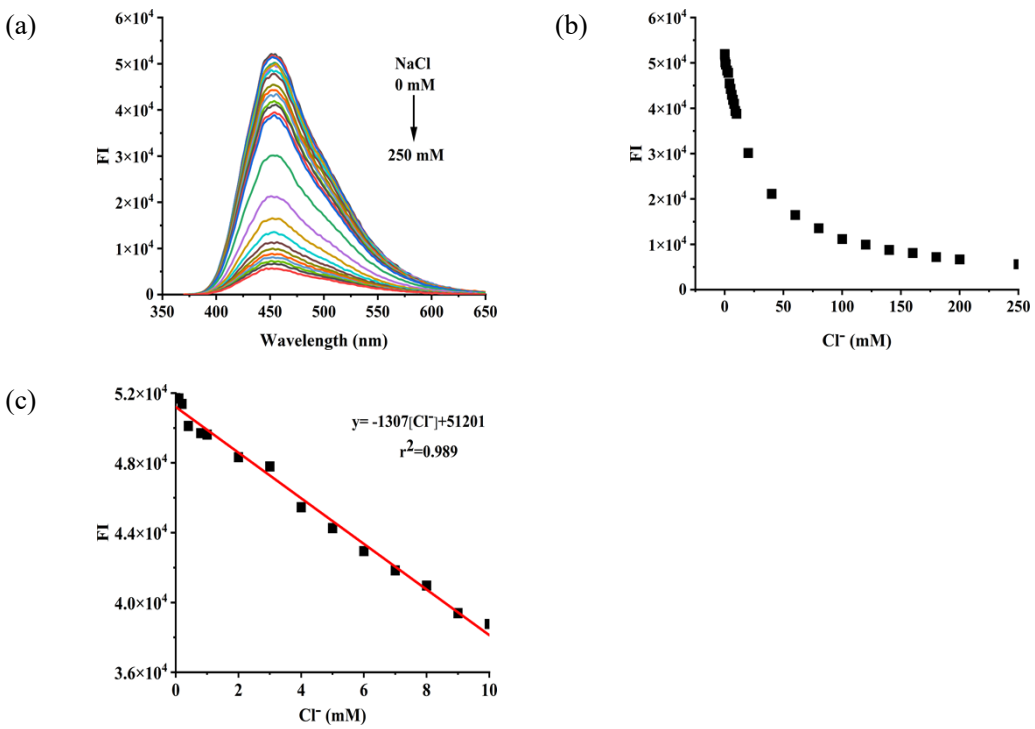

Fig. S8. (a) Fluorescence spectra of compound **1** (100 μM) in the presence of chloride anions of varying concentrations. (b) Plot of the fluorescent intensity of compound **1** (100 μM) against the concentrations of chloride anions. (c) Plot of the fluorescent intensity of compound **1** (100 μM) against the concentration of chloride anions in the range of 0.1-10 mM. The used media was 50 mM HEPES buffer (pH 7.4). $\lambda_{ex}/\lambda_{em}$ = 350/453 nm.

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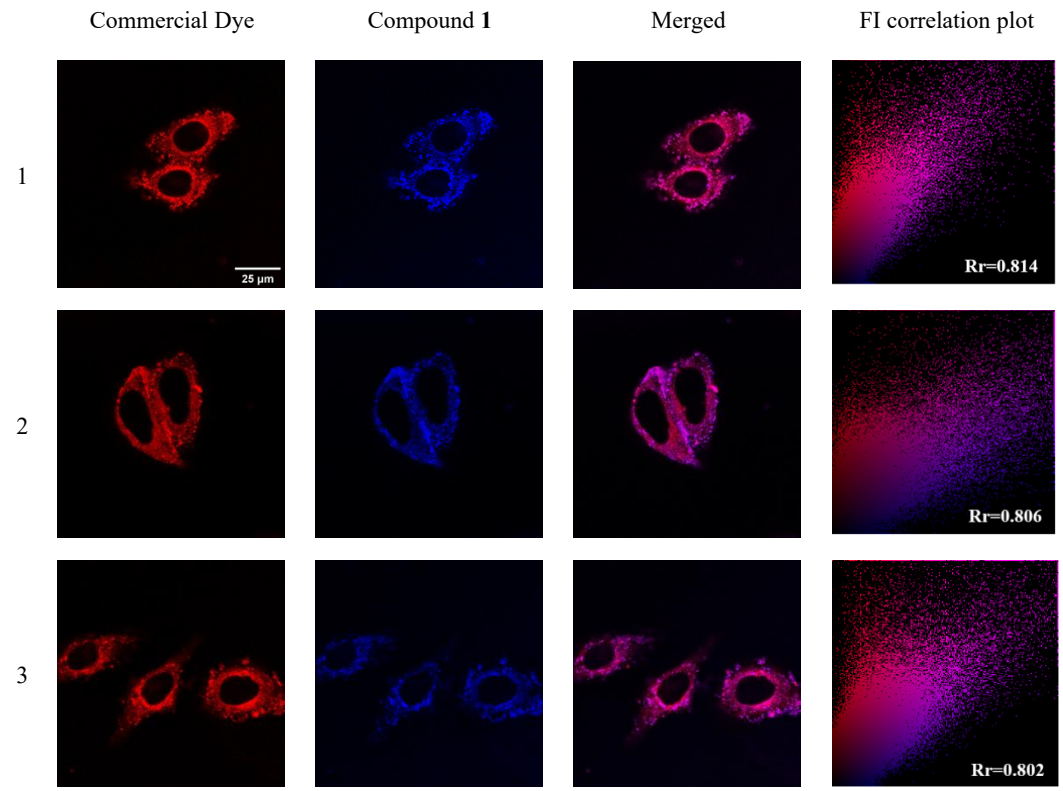

Fig. S9. Confocal imaging of HeLa cells co-stained by compound **1** (2 mM) with Golgi-Tracker Red (150 μg/mL).

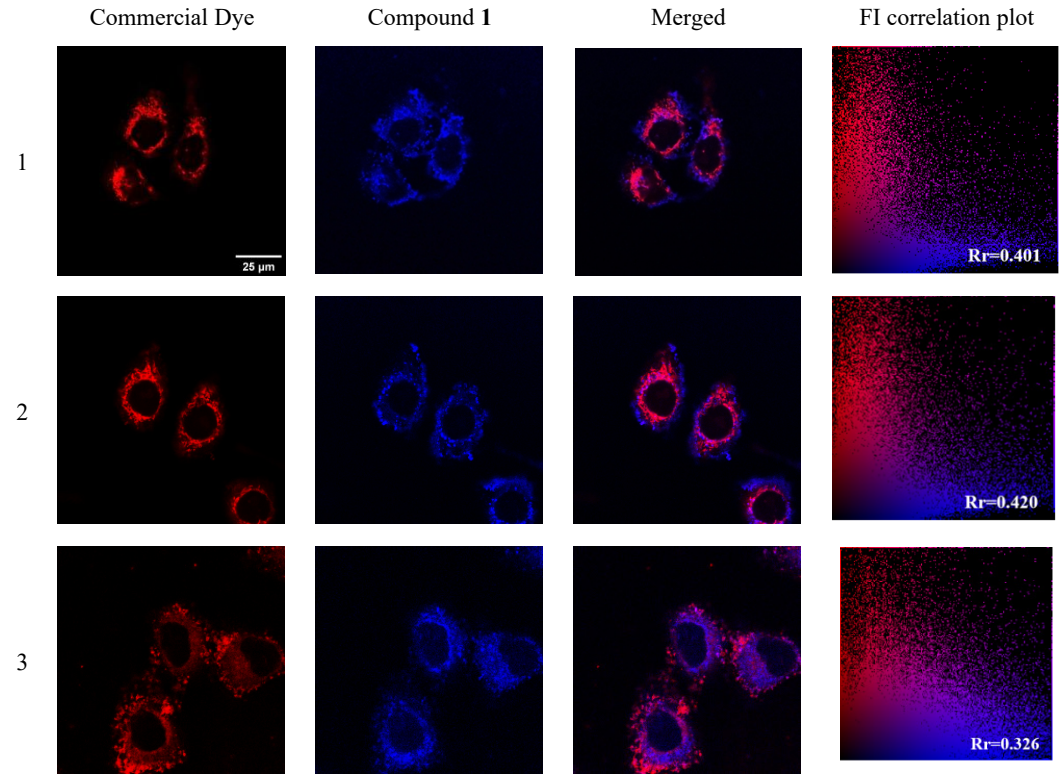

Fig. S10. Confocal imaging of HeLa cells co-stained by compound **1** (2 mM) with Mito-Tracker Red (1 μM).

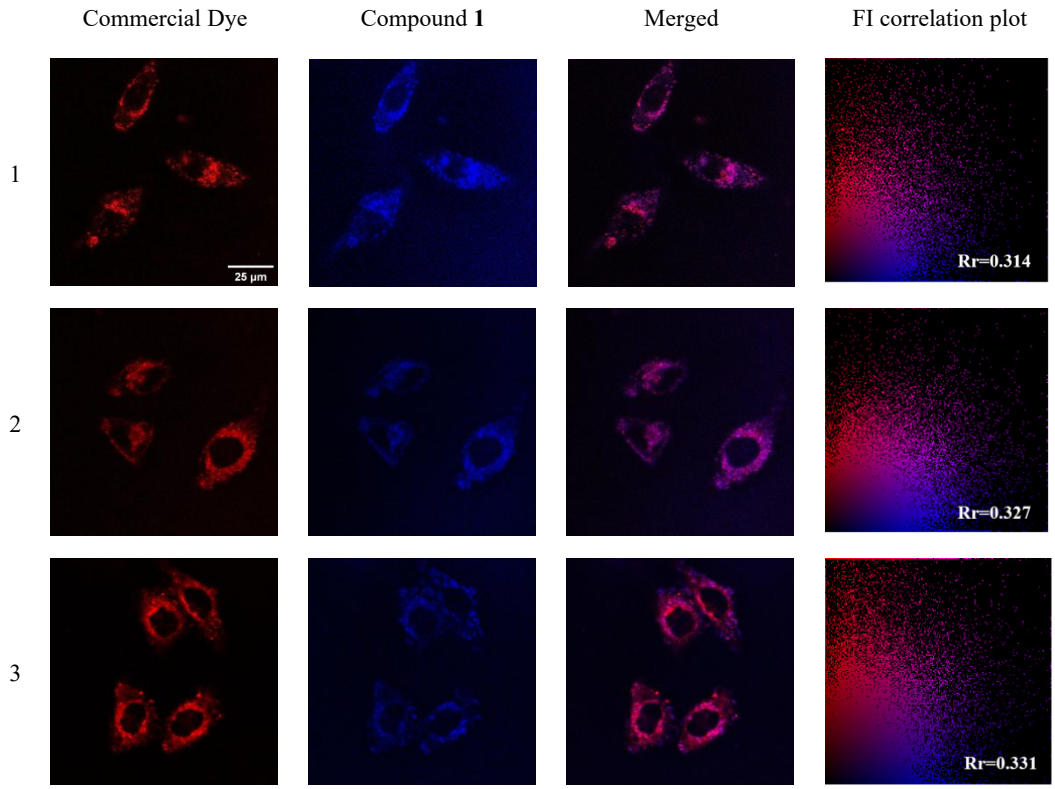

Fig. S11.Confocal imaging of HeLa cells co-stained by compound **1** (2 mM) with ER-Tracker Red (1 μM).

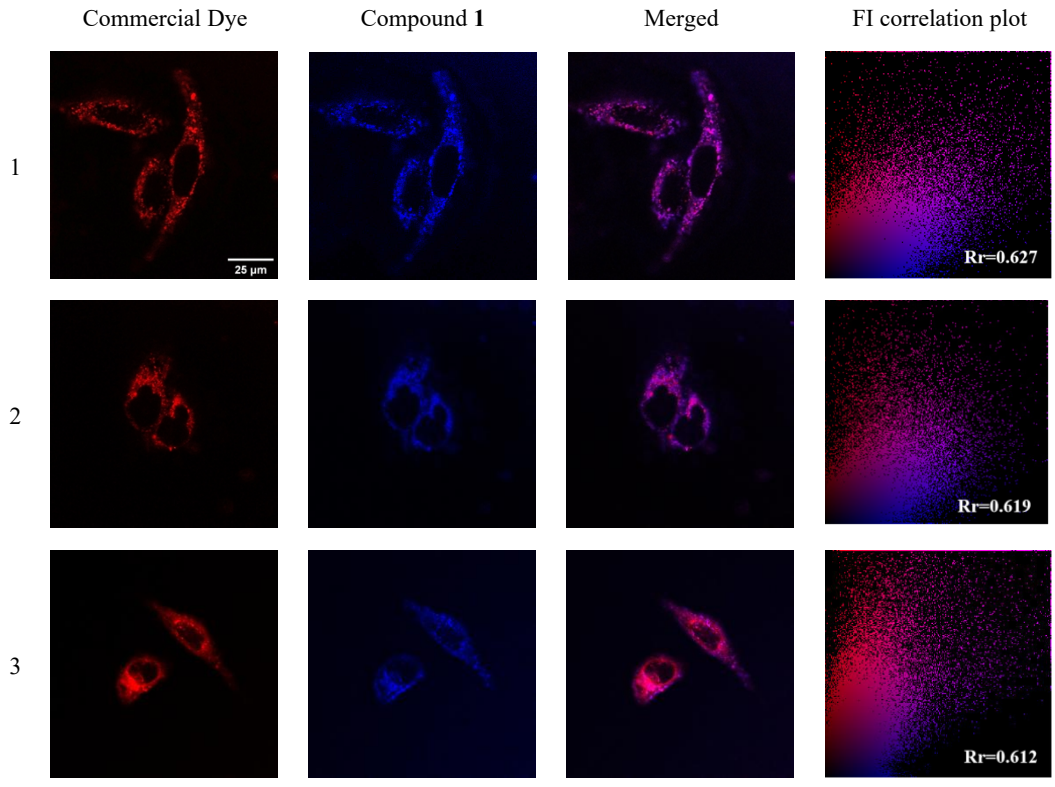

Fig. S12.Confocal imaging of HeLa cells co-stained by compound **1** (2 mM) with Lyso-Tracker Red (1 μM).

Table S1. Pearson correlation coefficient of compound **1** with four types of organelles [a]

	Golgi	Mito	ER	Lyso
1	0.814	0.401	0.314	0.627
2	0.806	0.420	0.327	0.619
3	0.802	0.326	0.331	0.612
Mean	0.807	0.382	0.324	0.619

[a] Data represent three independent replicate experiments.

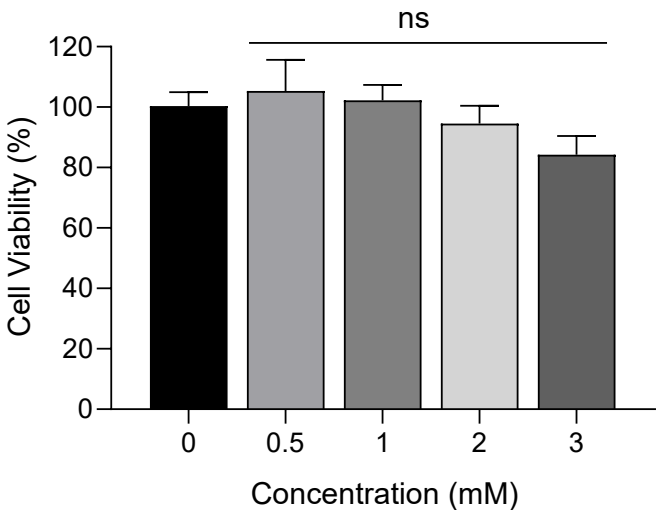

Fig. S13. Viability of HeLa cells treated with compound **1** of varying concentrations for 12 h. The data are represented as mean ± s.d. (n = 3). No significance (ns) *vs* the control group, *t*-test