Supporting Information for
Mitochondria-immobilized pH-Sensitive Off-On Fluorescent Probe

Min Hee Lee, a Nayoung Park, b Chunsik Yi, c Ji Hye Han, c Ji Hye Hong, d Kwang Pyo Kim, d Dong Hoon Kang, e Jonathan L. Sessler a,* Chulhun Kang, c,* and Jong Seung Kim b,*

aDepartment of Chemistry, The University of Texas at Austin, Austin, Texas 78712-1224, United States. bDepartment of Chemistry, Korea University, Seoul 136-701, Korea. cThe School of East-West Medical Science, Kyung Hee University, Yongin 446-701, Korea. dDepartment of Applied Chemistry, College of Applied Sciences, Kyung Hee University, Yongin 446-701, Korea. eDepartment of Life Science and Research Center for Cell Homeostasis, Ewha Womans University, Seoul 120-750, Korea.

*Corresponding authors: sessler@cm.utexas.edu (J. L. Sessler); kangch@khu.ac.kr (C. Kang); jongskim@korea.ac.kr (J. S. Kim)

Contents

1. Synthetic scheme
2. Synthesis
3. pKa of 2
4. Two-dimensional (2D) electrophoresis
5. 2D gel and enzymatic in-gel digestion
6. NanoLC-ESI-MS/MS analysis and database search
7. CCCP (m-chlorophenyl hydrazone) treatment
8. Intracellular pH calibration
9. Movie of confocal images
10. Additional experimental data
11. 1H-NMR, 13C-NMR and ESI-MS spectra
1. Synthetic scheme

**Figure S1.** Synthetic route leading to compound 1.

**Figure S2.** Synthetic route used to obtain compound 2.
2. Synthesis

Compounds 7, 8, 12, and 13 were prepared by adapting published procedures.\textsuperscript{1-3}

Synthesis of 1. Compound 6 (220 mg, 0.4 mmol), EDCI (260 mg, 1.3 mmol) and DMAP (55 mg, 0.4 mmol) were dissolved in DMF (10 mL). After stirring for 30 min, compound 7\textsuperscript{1} (172 mg, 0.4 mmol) was added. The reaction mixture was then stirred under nitrogen overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified over silica gel using dichloromethane/methanol (v/v, 43:4) as the eluent to yield 1 as a yellow solid (0.2 g, 60%). HRESI-MS \textit{m/z} (M\textsuperscript{+}) calc 779.29123, obs 779.29061.\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 8.88 (br t, 1 H); 8.43 (d, \textit{J} = 7.0 Hz, 1 H); 8.38-8.27 (m, 2 H); 7.81-7.61 (m, 15 H); 7.58 (t, \textit{J} = 7.8 Hz, 1 H); 7.41-7.34 (m, 4 H); 7.11 (d, \textit{J} = 8.1 Hz, 1 H); 4.57 (s, 2 H); 4.35 (t, \textit{J} = 7.2 Hz, 2 H); 3.81-3.58 (m, 6 H); 3.29 (br s, 4 H); 2.84 (br s, 4 H); 2.56 (t, \textit{J} = 7.3 Hz, 2 H). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): 172.2, 164.3, 163.8, 135.3, 133.8, 133.6, 132.5, 131.1, 130.8, 130.6, 130.3, 130.0, 128.9, 126.3, 125.8, 123.2, 118.5, 117.6, 116.9, 115.0, 62.4, 53.0, 52.9, 46.2, 36.6, 34.2, 33.2, 29.9, 22.8, 22.3 ppm.

Synthesis of 2. Compound 9 (250 mg, 0.6 mmol), EDCI (170 mg, 0.8 mmol) and DMAP (200 mg, 1.7 mmol) were dissolved in DMF (10 mL). After stirring for 30 min, compound 7\textsuperscript{1} (330 mg, 0.8 mmol) was added. The reaction mixture was stirred under nitrogen overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified over silica gel using dichloromethane/methanol (v/v, 20:1) as the eluent to yield 2 as a yellow solid (0.3 g, 67%). HRESI-MS \textit{m/z} (M\textsuperscript{+}) calc 731.31455, obs 731.31450.\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): 8.87 (br t, 1 H); 8.41-8.32 (m, 3 H); 8.01-7.51 (m, 16 H); 7.41-7.16 (m, 5 H); 7.10 (d, \textit{J} = 7.9 Hz, 1 H); 4.33 (br s, 2 H); 3.77-3.64 (m, 6 H); 3.24 (br s, 4 H); 2.75 (br s, 4 H); 2.56 (br s, 2 H). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): 171.9, 164.2, 163.7, 155.9, 135.2,
133.6, 133.5, 132.4, 130.9, 130.6, 130.3, 129.8, 128.3, 127.3, 126.1, 125.4, 123.1, 118.3, 117.4, 116.5, 114.7, 77.4, 77.1, 76.8, 62.9, 53.1, 36.4, 33.9, 33.0, 29.7, 22.2 ppm.

**Synthesis of 3.** 4-Bromo-1,8-naphthalic anhydride (3.0 g, 11.0 mmol), β-alanine tert-butyl ester hydrochloride (2.8 g, 15.4 mmol), and DMAP (1.6 mg, 13.0 mmol) were dissolved in ethanol (150 mL). The reaction mixture was stirred and heated at reflux for 10 h before being allowed to cool to room temperature. After removal of solvent under reduced pressure, the crude product was purified over silica gel using ethyl acetate/hexanes (v/v, 1:4) as the eluent. This gave 3 as a white solid (3.9 g, 89%). HRESI-MS m/z (M+H+) calc 406.04739, obs 406.04690; m/z (M+Na+) calc 428.02933, obs 428.02952. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 8.50 (d, J = 7.3 Hz, 1 H), 8.38 (d, J = 8.5 Hz, 1 H), 8.24 (d, J = 8.8 Hz, 1 H), 7.88 (d, J = 8.7 Hz, 1 H), 7.71 (t, J = 7.9 Hz, 1 H), 4.36 (t, J = 7.6 Hz, 2 H), 2.63 (t, J = 7.6 Hz, 2 H), 1.38 (s, 9 H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 170.3, 163.1, 133.1, 131.9, 131.1, 130.9, 130.2, 128.7, 127.9, 122.7, 121.9, 80.8, 36.3, 33.7, 28.0 ppm.

**Synthesis of 4.** Compound 3 (2.9 g, 7.2 mmol) and piperazine (6.2 g, 71.7 mmol) were dissolved in 2-methoxyethanol (300 mL). The reaction mixture was stirred and heated at reflux for 6 h before being allowed to cool to room temperature. After removal of solvent under reduced pressure, the crude product was purified by crystallization from ether/methanol. This yielded 4 as a yellow solid (2.5 g, 85%). HRESI-MS m/z (M+H+) calc 410.20743, obs 410.20741; m/z (M+Na+) calc 432.18938, obs 432.18926. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 8.58 (d, J = 7.7 Hz, 1 H); 8.52 (d, J = 8.0 Hz, 1 H); 8.42 (d, J = 7.9 Hz, 1 H); 7.69 (t, J = 7.9 Hz, 1 H); 7.22 (d, J = 7.2 Hz, 1 H); 4.43 (t, J = 7.6 Hz, 2 H); 3.30-3.19 (br d, 8 H); 2.67 (t, J = 7.6 Hz, 2 H); 2.27 (br s, 1 H); 1.42 (s, 9 H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 170.5, 164.2, 163.7, 156.3, 132.6, 131.1, 130.3, 129.8, 126.1, 125.6, 122.9, 116.4, 114.9, 80.6, 54.2, 46.1, 36.0, 33.8, 28.0 ppm.

**Synthesis of 5.** Compound 4 (2.4 g, 5.9 mmol), dichloro-p-xylene (10.3 g, 58.6 mmol), and K\(_2\)CO\(_3\) (0.4 g, 2.9 mmol) were dissolved in CH\(_2\)CN (300 mL). The reaction mixture was then stirred and heated at reflux for 12 h under nitrogen before being allowed to cool to room temperature. The white precipitates (excess dichloro-p-xylene and KCl) were filtered off and the yellow colored filtrate was reduced in volume on the rotary evaporator. It was then purified over silica gel using dichloromethane/methanol (v/v, 30:1) as the eluent. This gave 5 as a yellow solid (2.5 g, 78%). ESI-MS m/z (M+H+) calc 548.23106, obs 548.23081; (M+Na\(^+\)) calc 570.21301, obs 570.21276. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 8.51-8.49 (dd, J = 7.3, 1.1 Hz, 1 H); 8.45 (d, J = 8.1 Hz, 1 H); 8.37-8.34 (dd, J = 8.5, 1.2 Hz, 1 H); 7.65-7.60 (dd, J = 8.4, 7.3 Hz, 1 H); 7.36 (s, 4 H); 7.15 (d, J = 8.1 Hz, 1 H); 4.57 (s, 2 H); 4.39 (t, J = 7.6 Hz, 2 H); 3.64 (s, 2 H); 3.26 (br s, 4 H); 2.75 (br s, 4 H); 2.65 (t, J = 7.6 Hz, 2 H); 1.41 (s, 9 H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 170.6, 164.2, 163.7, 156.0, 138.2, 136.8, 132.6, 131.1, 130.4, 129.8, 129.5, 128.6, 126.0, 125.6, 122.9, 116.3, 114.8, 80.6, 62.6, 53.1, 53.01, 46.1, 36.0, 33.9, 28.0 ppm.

**Synthesis of 6.** A solution of TFA/DCM (v/v, 5:1) was added to compound 5 (1.2 g, 2.2 mmol). After 12 h of stirring, the volatiles were removed under reduced pressure to yield 6 as a yellow solid (1.1 g, 99%). The identity of this crude product was confirmed by \(^1\)H NMR and ESI-MS analysis before being used directly for the next reaction. HRESI-MS m/z (M\(^+\)) calc 491.2, obs 492.2 (M+H\(^+\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)/CD\(_2\)OD): δ 8.51-8.49 (dd, J = 7.3, 0.9 Hz, 1 H); 8.46 (d, J = 8.0 Hz, 1 H); 8.35-8.32 (dd, J = 8.5, 1.0 Hz, 1 H); 7.74-7.70 (dd, J = 8.4, 7.4 Hz, 1 H); 7.52-7.48 (m, 4 H); 7.31 (d, J = 8.1 Hz, 1 H); 4.59 (s, 2 s); 4.50 (br s, 8 H); 4.40-4.37 (m, 4 H); 2.69 (t, J = 7.6 Hz, 2 H).

**Synthesis of 9.** Compound 8\(^2\) (1.0 g, 3.6 mmol) and 1-benzylpiperazine (944 μL, 5.4 mmol) were dissolved in 2-methoxyethanol (50 mL). The reaction mixture was then stirred and
heated at reflux for 6 h before being allowed to cool to room temperature. After removal of solvent under reduced pressure, the resulting crude product was purified over silica gel using dichloromethane/methanol (v/v, 35:1) as the eluent. This yielded 9 as a yellow solid (1.0 g, 62%). HRESI-MS m/z (M+H\(^+\)) calc 444.19178, obs 444.19199; m/z (M+Na\(^+\)) calc 466.17373, obs 466.17379. \(^1\)H NMR (400 MHz, CDCl\(_3)/CD_2OD): \(\delta\) 8.45 (dd, \(J = 7.3, 1.1\) Hz, 1 H); 8.38 (dd, \(J = 8.3, 1.5\) Hz, 2 H); 7.66 (m, 1 H); 7.38-7.25 (m, 5 H); 7.23 (d, \(J = 8.2\) Hz, 1 H); 4.35 (t, \(J = 7.6\) Hz, 2 H); 3.83 (s, 2 H); 3.24 (br s, 4 H); 2.96 (br s, 4 H); 2.64 (t, \(J = 7.6\) Hz, 2 H). \(^13\)C NMR (100 MHz, CDCl\(_3\)): 175.0, 164.3, 163.8, 155.8, 135.5, 132.7, 131.1, 130.3, 129.9, 129.8, 127.8, 126.1, 125.7, 123.1, 116.5, 115.1, 52.4, 52.1, 36.2, 33.2, 30.9 ppm.

**Synthesis of 10.** Compound 3 (200 mg, 0.5 mmol) and an excess of diethylamine (3 mL) were dissolved in 2-methoxyethanol (10 mL). The reaction mixture was stirred and heated at reflux overnight before being allowed to cool to room temperature. After removal of the solvent under reduced pressure, the crude product was extracted with dichloromethane/water several times. The combined organic phases were dried over Na\(_2\)SO\(_4\) and filtered. After removal of solvent, the crude product obtained from the filtrate was purified over silica gel using ethyl acetate/hexanes (v/v, 1:5) as the eluent. This yielded 10 as a yellow solid (170 mg, 89%). HRESI-MS m/z (M+Na\(^+\)) calc 419.19430, obs 419.19410. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.58 (dd, \(J = 7.3, 1.2\) Hz, 1 H); 8.54-8.42 (m, 2 H); 7.67 (dd, \(J = 8.5, 7.3\) Hz, 1 H); 7.22 (d, \(J = 8.2\) Hz, 1 H); 4.45 (t, \(J = 7.7\) Hz, 2 H); 3.43 (q, \(J = 7.1\) Hz, 4 H); 2.70 (t, \(J = 7.7\) Hz, 2 H); 1.45 (s, 9 H); 1.19 (t, \(J = 7.1\) Hz, 6 H). \(^13\)C NMR (100 MHz, CDCl\(_3\)): 170.8, 164.5, 164.1, 155.5, 132.2, 131.2, 130.1, 127.4, 125.1, 123.0, 116.9, 115.5, 81.1, 47.3, 36.2, 33.9, 28.0, 12.3 ppm.

**Synthesis of 11.** A solution of TFA/DCM (v/v, 5:1) was added to compound 10 (170 mg, 0.4 mmol). After stirring for 12 h, the volatiles were removed under reduced pressure to yield de-tert-butylated 10 as a yellow solid (200 mg, 67%). The identity of this latter crude product was confirmed by silica-coated TLC and \(^1\)H NMR spectral analyses. It was then used directly for the next reaction. Here, de-tert-butylated 10 (140 mg, 0.4 mmol), EDCI (236 mg, 1.2 mmol) and DMAP (75 mg, 0.6 mmol) were dissolved in DMF (5 mL). After 30 min of stirring, compound 7\(^1\) (238 mg, 0.6 mmol) was added. The reaction mixture was further stirred under nitrogen overnight at room temperature. Solvent was removed and the residue was purified over silica gel using dichloromethane/methanol (v/v, 40:3) as the eluent. This yielded 11 as a yellow solid (0.2 g, 60%). HRESI-MS m/z (M\(^+\)) calc 628.27430, obs 628.27430. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 9.01 (br s, 1 H); 8.49 (d, \(J = 7.2\) Hz, 1 H); 8.43 (dd, \(J = 8.3, 4.1\) Hz, 2 H); 7.88-7.65 (m, 15 H); 7.61 (t, \(J = 7.9\) Hz, 1 H); 7.17 (t, \(J = 8.1\) Hz, 1 H); 4.40 (t, \(J = 7.3\) Hz, 2 H); 3.88-3.78 (m, 2 H); 3.79-3.69 (m, 2 H); 3.40 (q, \(J = 7.0\) Hz, 4 H); 2.60 (t, \(J = 7.4\) Hz, 2 H); 1.16 (t, \(J = 7.0\) Hz, 6 H). \(^13\)C NMR (100 MHz, CDCl\(_3\)): 172.3, 164.6, 163.9, 155.1, 135.3, 133.6, 132.0, 130.9, 130.8, 127.3, 125.1, 1223.0, 118.4, 117.6, 116.7, 115.7, 47.3, 36.7, 34.5, 33.1, 23.0, 22.0, 12.5 ppm.

### 3. pKa of 2

The pKa value of 2 was estimated from the changes in fluorescence intensity observed at various pH values using the relationship, \(\log([I_{\text{max}}-I]/I_{\text{min}}]) = \mathrm{pH} - \mathrm{pK}a\), where \(I_{\text{max}}, I_{\text{min}}\) and I are the maximum, minimum, and observed fluorescence intensities at a given pH, respectively. The pKa value (y-intercept) for 2 (\(\mathrm{pK}a = 6.186 \pm 0.04999\)) was derived from a plot of pH vs \(\log([I_{\text{max}}-I]/I_{\text{min}}])\). This plot is shown in Figure S5. Other information is available in the figure captions.
4. Two-dimensional (2D) electrophoresis
HeLa cells were seeded on 6-well plates (1×10^5 cells/well) and treated with culture media containing 5 µM of the compound under study (either 1, 2, or 3) for 0, 1, 3, and 5 h at 37 °C, respectively. The HeLa cells were harvested, washed three times in phosphate buffered saline (PBS) and lysed in RIPA (Radio-Immunoprecipitation Assay) buffer. The protein contents were determined according to Bradford’s method. The same volume of 100% acetone was added to the lysed samples. After mild vortexing, the samples were incubated at -20 °C for at least 2 h. The samples were then centrifuged for 10 min at 12000 rpm and the supernatants were decanted carefully. The pellets obtained in this way were dried briefly and resuspended in rehydration buffer (8 M urea, 2 M thiourea, 2% (w/v) CHAPS (3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate), 1% (w/v) DTT (dithiothreitol), 1% (v/v) ampholyte (pH 3-10) and 1 µl/mL protease inhibitor cocktail). The resulting solutions were applied to an isoelectric focusing strip (pH 5-8 gradient, 7 cm Bio-Rad). The strips were rehydrated at 50 V for 13 h and focused at up to 43,107 Vh. The temperature was maintained at 20 °C during isoelectric focusing. After isoelectric focusing, the strips were treated with equilibration solution (50 mM Tris-HCl pH 8.8, 6 M urea, 30% (w/v) glycerol, 2% (w/v) SDS (sodium dodecyl sulfate), 1% (w/v) DTT, 0.001% (w/v) bromophenol blue) for 15 min. They were then with the same buffer lacking DTT but containing 2.5% (w/v) iodoacetamide for 15 min. The samples were subjected to two-dimensional (2D) electrophoresis using a 10% gel in accord with Laemmli’s method. Fluorescent gel imaging was carried out using a Typhoon 9400 scanner with excitation at 457 nm (Blue Laser), and emission 520 nm band-pass filter, photomultiplier tube (PMT) 800 V and 100 microm pixel resolution. Gel staining was performed using a modified colloidal CBB (coomassie brilliant blue) G250 staining method. The staining gels were destained with doubly distilled water for 24 h. The images were obtained with a UMAX PowerLook 2100XL scanner (Willich, Germany).

5. 2D gel and enzymatic in-gel digestion
Samples were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) on a 4-20% gradient Novex Tris-Glycine gel (Invitrogen, Carlsbad, CA, USA) followed by staining with GelCode Blue Stain Reagent (Pierce, Rockford, IL). The stained gel was destained with treating with 50% acetonitrile in 50 mM NH₄HCO₃ (ammonium bicarbonate, ABC). The destained gel pieces were dehydrated in 100% acetonitrile and digested with sequencing grade modified trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate. Digested peptides were extracted from the gel using 5% formic acid in 50% acetonitrile. The extracts were dried and purified using a C18 spin column (Millipore, Temecula, CA, USA).

6. NanoLC-ESI-MS/MS analysis and database search
The extracted tryptic peptides were analyzed using a Q-Exactive mass spectrometer (Thermo Fisher Scientific) coupled with an Easy-nanoLC system (Thermo Fisher Scientific). Tryptic peptides were applied to the heated analytic column (EASY-Spray column, Thermo Scientific, 2 µm particle size, 100 Å pore size, 75 µm id × 50 cm length). Samples were resolved with a linear gradient of Solvent B (0.1% formic acid in acetonitrile) with Solvent A (0.1% formic acid in water); 5–50% over 76 min, 50-90% over 12 min at a flow rate of 350 nL/min. The separated peptide ions eluted from the analytic column were injected into the mass spectrometer at an electrospray voltage of 2.1 kV. All MS/MS spectra were acquired in
a data-dependent mode tuned for fragmentation of the ten most abundant peaks from the full MS scan with 27% normalized collision energy. The dynamic exclusion duration was set at 20 s and the isolation mass width was 2 Da. MS spectra were acquired over a mass range of 300-2000 m/z and a resolution of 70,000. Higher-energy collisional dissociation (HCD) peptide fragments were acquired at 27% of the normalized collision energy. MS/MS resolution was 17,500. The acquired MS/MS spectra were referenced to the Universal Protein Resource human protein database (Uniprot, http://www.uniprot.org/) and validated with the Proteome Discoverer 1.4 program (Thermo Fisher Scientific) using the following search parameters: Enzyme specificity/trypsin, precursor ion mass tolerances of 10 ppm, carbamidomethylation on cysteine as a fixed modification, and oxidation of methionine as a variable modification. The False Discovery Rate (FDR), determined by using a reversed database was set to 1%. In this way, we obtained a list of peptides established at greater than 95% probability according to the Peptide Prophet algorithm. When at least two unique peptides are used, peptide identification with Protein Prophet is considered reliable to the 99% confidence level.

7. CCCP (m-chlorophenyl hydrazone) treatment
HeLa cells were separately pretreated with media containing 1 and 2 (5.0 µM each) for 5 h. The media were replaced with PBS containing CCCP (10.0 µM) and incubated for either 1 h or 6 h at 37 °C. The cells were washed three times with PBS. Fluorescent confocal images were then recorded using an excitation wavelength of 488 nm and band-path emission filter 500–640 nm.

8. Intracellular pH calibration
HeLa cells were pretreated with media containing 1 (5.0 µM) and MitoTracker Red (MTR) (0.1 µM) for 5 h at 37 °C. The cells were then washed with pre-warmed PBS containing 3.7% formaldehyde and incubated for 15 min at 37 °C. The fixed cells were washed with fresh PBS several times and incubated with high K+ buffer (30 mM NaCl, 120 mM KCl, 1 mM CaCl2, 0.5 mM MgSO4, 1 mM NaH2PO4, 5 mM glucose, 20 mM HEPES, 20 mM NaOAc) at various pH values (5–8). After 5 min, the green (probe 1) and red (MTR) channel images were recorded with a confocal microscope. A pH calibration curve (Igreen/Ired vs pH) was then constructed.

9. Movie of confocal images
**Intact cell:** HeLa cells were pretreated with probe 1 (5.0 µM) for 5 h at 37 °C before LysoTracker Red (LTR) (1.0 µM) was added. After incubating for 10 min at 37 °C, the images were collected at 15 sec intervals ranging from baseline (t = 0) to 435 sec. **Nutrient-deprived cell:** HeLa cells were pretreated with probe 1 (5.0 µM) for 5 h at 37 °C. The medium was then washed and replaced with serum-free Krebs-Ringer-HEPES buffer (KRH: 115 mM NaCl, 5 mM KCl, 1 mM KH2PO4, 1.2 mM MgSO4, and 2 mM CaCl2, 25 mM HEPES) at pH 7.4 containing glucagon (1.0 µM) and pepstatin A (7.5 µM). After 1.5 h at 37 °C, the nutrient-deprived cells were treated with LysoTracker Red (LTR) (1.0 µM) for 10 min. The images were then collected at 15 sec intervals ranging from baseline (t = 0) to 435 sec.
10. Additional experimental data

**Figure S5.** Plot of pH vs \( \log[(I_{\text{max}}-I)/(I-I_{\text{min}})] \), where \( I \) is the observed fluorescence intensity of 2 at 525 nm upon excitation at 407 nm. The y-intercept is the \( pK_a \) value (6.186 ± 0.04999) of 2.

**Figure S6.** Absorption (a) and fluorescence (b) spectra of 11 (10.0 and 1.0 μM, respectively) at different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0). All data were obtained using an excitation wavelength of 450 nm in 33 mM of buffer solution containing 1% (v/v) DMSO at 37 °C.
Figure S7. Absorption (a) and fluorescence (b) spectra of 13 (10.0 μM) at different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0). All data were obtained using an excitation wavelength of 434 nm in 33 mM of buffer solution containing 1% (v/v) DMSO at 37 °C.

Figure S8. Fluorescence spectra of 2 (1.0 μM) in the presence of 100 μM of metal ions (Na⁺, K⁺, Ca²⁺, Zn²⁺, Mg²⁺, Mn²⁺, Cu²⁺, Fe²⁺, Fe³⁺, as their chloride salts), 5.0 mM of thiols (GSH, Cys, Hcy), and 100 μM of H₂O₂ in PBS solution (20 mM, pH 7.4, 37 °C) containing 1% (v/v) DMSO. All data were obtained using an excitation wavelength of 407 nm.
Figure S9. Colocalization experiments using 2 and MitoTracker Red (MTR) in HeLa cells. The cells were incubated with 2 (5.0 µM) for 10 min at 37 °C and the medium was replaced with fresh medium containing MTR (0.5 µM) and incubated for 10 min. Images for 2 (a) and MTR (b) were then recorded using excitation wavelengths at 488 nm and 633 nm, and recording over the 500–550 nm and 700–750 nm spectral regions, respectively. Panels (c) and (d) show a merged image of (a) and (b) and the corresponding bright field image, respectively.

Figure S10. Colocalization experiments using 5 and MitoTracker Red (MTR) in HeLa cells. The cells were incubated with 5 (5.0 µM) for 10 min at 37 °C and the medium was replaced with fresh medium containing MTR (0.5 µM) and incubated for 10 min. Images for 5 (a) and MTR (b) were then recorded using excitation wavelengths of 488 nm and 633 nm, and recording over the 500–550 nm and 700–750 nm spectral regions, respectively. Panels (c) and (d) show a merged image of (a) and (b) and the corresponding bright field image, respectively.
Figure S11. Cell viability of 1 in HeLa cells. The cells were seeded at 3 \times 10^4 cells/well on a 96-well plate. When 80% confluency was reached, the cells were treated with media containing 1 (5.0 µM) for 5 h, and MTT assay was then performed. The data are based on the average and show the standard deviation (n = 6).

Figure S12. SDS-PAGE analysis of proteins collected from the control (N) and cells treated with compounds 1, 2, and 5 for 5 h, or cells treated with 1 for 1 h, 3 h, and 5 h at 37 °C, respectively. M represents a size marker. (a) Typhoon images showing fluorescent bands obtained using an excitation wavelength of 457 nm. (b) All bands were visualized by CBB staining.
Table S1. List of proteins obtained from the fluorescent bands (see also Table S2). The 18 proteins were confidently identified from triplicate analysis of samples prepared as described in the experimental procedures. The reported proteins were identified using Sequest with FDR < 0.01.

| Description                                                                 | Spectral Counts |
|-----------------------------------------------------------------------------|-----------------|
|                                                                             | 1st  | 2nd  | 3rd  |
| Protein disulfide-isomerase A6                                              | 37   | 8    | 64   |
| ATP synthase subunit beta, mitochondrial                                   | 34   | 16   | 70   |
| Actin, cytoplasmic 1                                                        | 28   | 5    | 65   |
| 26S protease regulatory subunit 6A                                           | 27   | 14   | 55   |
| 60 kDa heat shock protein, mitochondrial                                    | 25   | 3    | 8    |
| Eukaryotic initiation factor 4A-I                                           | 17   | 7    | 5    |
| Ribonuclease inhibitor                                                      | 17   | 13   | 2    |
| Tubulin beta chain                                                          | 17   | 12   | 83   |
| Vimentin                                                                    | 16   | 27   | 107  |
| Tubulin beta-4B chain                                                       | 14   | 8    | 82   |
| Alpha-enolase                                                               | 12   | 7    | 5    |
| Eukaryotic translation initiation factor 3 subunit F                        | 11   | 12   | 16   |
| Elongation factor 1-alpha 1                                                 | 10   | 11   | 3    |
| PRKAR2A protein                                                             | 4    | 2    | 6    |
| Endoplasmic reticulum resident protein 44                                   | 3    | 8    | 3    |
| Heterogeneous nuclear ribonucleoproteins C1/C2 (Fragment)                   | 3    | 6    | 30   |
| 40S ribosomal protein SA (Fragment)                                         | 2    | 3    | 2    |
| Annexin                                                                     | 2    | 2    | 2    |
Table S2. The list of identified proteins from the fluorescent bands on the 2D gel.

| Protein name | Gene Name | Accession | Peptide sequence | Total Precursor Intensity | Protein identification probability | Percentage sequence coverage |
|--------------|-----------|-----------|-----------------|---------------------------|----------------------------------|-------------------------------|
| 14-3-3 protein zeta-delta | VHL2 | 14332 | (list of peptides) | 0.1 | 100.0% | 16.0% |
| 26S protease regulatory subunit 4 | PRS1 | 21552 | (list of peptides) | 0.1 | 100.0% | 15.0% |
| 26S protease regulatory subunit 6A | PRS6A | 21552 | (list of peptides) | 0.1 | 100.0% | 15.0% |
| 26S protease regulatory subunit 6B | PRS6B | 21552 | (list of peptides) | 0.1 | 100.0% | 15.0% |
| 34 kDa COXIV subunit | HAD1 | B42W1 | (list of peptides) | 0.1 | 100.0% | 16.0% |
| 40S ribosomal protein S1 | RPS4PS | A6E39 | (list of peptides) | 0.1 | 100.0% | 16.0% |
| Gene                        | Start | End  | Amino Acid Length | Identity | Similarity |
|-----------------------------|-------|------|-------------------|----------|------------|
| Actin, alpha skeletal muscle | ACTA1 | ACTB | 335               | 100.00%  | 52.5%      |
| Actin, beta skeletal muscle  | ACTB  | ACTB | 335               | 100.00%  | 52.5%      |
| Alpha-actinin              | ANX1  | ANX1 | 194               | 100.00%  | 52.5%      |
| ATP synthase subunit beta, mitochondrial | ATP5B | ATP5B | 210               | 100.00%  | 52.5%      |
| 60kDa heat shock protein, mitochondrial | HSPO1 | CHB6 | 450               | 100.00%  | 52.5%      |
| 78kDa glucose-regulated protein | HSFA5 | GRP78 | 190               | 100.00%  | 52.5%      |

**S14**
| Protein                         | Accession | Description                           | Score | E-value |
|--------------------------------|-----------|---------------------------------------|-------|---------|
| cAMP-dependent protein kinase type I-alpha regulatory subunit | PRKAR1A   | KAP9                                 | 265   | 100.0   |
|                                |           | MATK                                 | 245   | 100.0   |
|                                |           | JSNEDD4                              | 306   | 100.0   |
|                                |           | SNEPETFW                               | 306   | 100.0   |
|                                |           | YLR4P3C104W3                        | 306   | 100.0   |
|                                |           | VSL101K                              | 358   | 100.0   |
|                                |           | VSL101K                              | 233   | 100.0   |
|                                |           | AAXAVV                                | 348   | 100.0   |
|                                |           | AATTVVAVLQGVVDLR                     | 218   | 100.0   |
| cAMP-dependent protein kinase type II-alpha regulatory subunit | PRKAR2A   | KAP2                                 | 162   | 100.0   |
|                                |           | VSL101K                              | 304   | 100.0   |
|                                |           | GYTVLIVK                               | 182   | 100.0   |
|                                |           | GYTVLIVK                               | 182   | 100.0   |
|                                |           | YSL101K                              | 130   | 100.0   |
|                                |           | MFY5V5VLQGVVDLR                     | 392   | 100.0   |
|                                |           | CF408EFH                               | 396   | 100.0   |
|                                |           | CBVD4                                | 222   | 100.0   |
|                                |           | NVF1                                 | 120   | 100.0   |
|                                |           | NF31                                 | 120   | 100.0   |
|                                |           | THEREBF                               | 913   | 100.0   |
|                                |           | SLEAVYAVVYLYVYV                        | 165   | 100.0   |
|                                |           | LQTEVYV                              | 230   | 100.0   |
|                                |           | NVF31                                | 325   | 100.0   |
|                                |           | THEREBF                               | 185   | 100.0   |
|                                |           | NVF31                                | 302   | 100.0   |
|                                |           | NVF31                                | 162   | 100.0   |
|                                |           | NVF31                                | 149   | 100.0   |
|                                |           | AMITTFYFV                               | 267   | 100.0   |
|                                |           | AVTETTVYV                                | 298   | 100.0   |
|                                |           | ENLTVVYVVDLQGS                      | 380   | 100.0   |
|                                |           | ENLTVVYVVDLQGS                      | 126   | 100.0   |
|                                |           | LLQ101K                              | 157   | 100.0   |
|                                |           | LLQ101K                              | 157   | 100.0   |
|                                |           | LLQ101K                              | 157   | 100.0   |
|                                |           | LLQ101K                              | 177   | 100.0   |
|                                |           | LLQ101K                              | 177   | 100.0   |
|                                |           | LLQ101K                              | 283   | 100.0   |
|                                |           | LLQ101K                              | 231   | 100.0   |
|                                |           | LLQ101K                              | 195   | 100.0   |
|                                |           | LLQ101K                              | 122   | 100.0   |
|                                |           | LLQ101K                              | 309   | 100.0   |
|                                |           | LLQ101K                              | 268   | 100.0   |
|                                |           | LLQ101K                              | 319   | 100.0   |
|                                |           | LLQ101K                              | 135   | 100.0   |
|                                |           | LLQ101K                              | 256   | 100.0   |
|                                |           | LLQ101K                              | 101   | 100.0   |
|                                |           | LLQ101K                              | 147   | 100.0   |
|                                |           | LLQ101K                              | 147   | 100.0   |
|                                |           | LLQ101K                              | 636   | 100.0   |
|                                |           | LLQ101K                              | 476   | 100.0   |
|                                |           | LLQ101K                              | 202   | 100.0   |
|                                |           | LLQ101K                              | 415   | 100.0   |
|                                |           | LLQ101K                              | 138   | 100.0   |
|                                |           | LLQ101K                              | 401   | 100.0   |
|                                |           | LLQ101K                              | 402   | 100.0   |
|                                |           | LLQ101K                              | 182   | 100.0   |
|                                |           | LLQ101K                              | 174   | 100.0   |
|                                |           | LLQ101K                              | 286   | 100.0   |
|                                |           | LLQ101K                              | 286   | 100.0   |
|                                |           | LLQ101K                              | 190   | 100.0   |
|                                |           | LLQ101K                              | 190   | 100.0   |
|                                |           | LLQ101K                              | 191   | 100.0   |
|                                |           | LLQ101K                              | 104   | 100.0   |
|                                |           | LLQ101K                              | 104   | 100.0   |
|                                |           | LLQ101K                              | 162   | 100.0   |
|                                |           | LLQ101K                              | 162   | 100.0   |
|                                |           | LLQ101K                              | 346   | 100.0   |
|                                |           | LLQ101K                              | 314   | 100.0   |
|                                |           | LLQ101K                              | 191   | 100.0   |
|                                |           | LLQ101K                              | 102   | 100.0   |
|                                |           | LLQ101K                              | 240   | 100.0   |
|                                |           | LLQ101K                              | 344   | 100.0   |
|                                |           | LLQ101K                              | 100   | 100.0   |
|                                |           | LLQ101K                              | 239   | 100.0   |
|                                |           | LLQ101K                              | 370   | 100.0   |
|                                |           | LLQ101K                              | 238   | 100.0   |
|                                |           | LLQ101K                              | 284   | 100.0   |
|                                |           | LLQ101K                              | 147   | 100.0   |
|                                |           | LLQ101K                              | 178   | 100.0   |
|                                |           | LLQ101K                              | 248   | 100.0   |
|                                |           | LLQ101K                              | 325   | 100.0   |
|                                |           | LLQ101K                              | 119   | 100.0   |
|                                |           | LLQ101K                              | 214   | 100.0   |
|                                |           | LLQ101K                              | 277   | 100.0   |
| Protein Name                                      | Accession | MW (kDa) | PDB  |
|--------------------------------------------------|-----------|----------|------|
| Enractin translation initiation factor 2 subunit 2| EIF2B2    | 173      | 12.8 |
|                                                  | IF2B     | 246      | 12.8 |
|                                                  | LYS      | 297      | 12.8 |
|                                                  | PHF1     | 286      | 12.8 |
|                                                  | PHF2     | 217      | 12.8 |
|                                                  | PHF3     | 215      | 25.0 |
|                                                  | PHF4     | 258      | 25.0 |
|                                                  | PHF5     | 307      | 25.0 |
|                                                  | PHF6     | 247      | 25.0 |
|                                                  | PHF7     | 237      | 25.0 |
|                                                  | PHF8     | 116      | 38.6 |
|                                                  | PHF9     | 239      | 39.3 |
|                                                  | PHF10    | 127      | 39.5 |
|                                                  | PHF11    | 262      | 11.9 |
|                                                  | PHF12    | 262      | 11.9 |
|                                                  | PHF13    | 310      | 14.0 |
|                                                  | PHF14    | 160      | 17.3 |
|                                                  | PHF15    | 194      | 17.3 |
|                                                  | PHF16    | 273      | 17.3 |
|                                                  | PHF17    | 101      | 13.8 |
|                                                  | PHF18    | 66       | 13.8 |
|                                                  | PHF19    | 387      | 13.8 |
|                                                  | PHF20    | 154      | 13.8 |
|                                                  | PHF21    | 396      | 13.8 |
|                                                  | PHF22    | 653      | 14.6 |
|                                                  | PHF23    | 482      | 14.6 |
|                                                  | PHF24    | 263      | 18.8 |
|                                                  | PHF25    | 317      | 17.0 |
|                                                  | PHF26    | 180      | 17.0 |
|                                                  | PHF27    | 151      | 10.7 |
|                                                  | PHF28    | 136      | 46.5 |
|                                                  | PHF29    | 205      | 46.5 |
|                                                  | PHF30    | 349      | 21.2 |
|                                                  | PHF31    | 283      | 19.8 |
|                                                  | PHF32    | 341      | 19.8 |
|                                                  | PHF33    | 340      | 19.8 |
|                                                  | PHF34    | 266      | 19.8 |
|                                                  | PHF35    | 133      | 11.7 |
|                                                  | PHF36    | 174      | 11.7 |
|                                                  | PHF37    | 174      | 11.7 |
|                                                  | PHF38    | 119      | 11.7 |
|                                                  | PHF39    | 312      | 13.8 |
|                                                  | PHF40    | 221      | 13.8 |
|                                                  | PHF41    | 232      | 13.8 |
|                                                  | PHF42    | 347      | 13.8 |
|                                                  | PHF43    | 348      | 13.8 |
|                                                  | PHF44    | 336      | 14.8 |
|                                                  | PHF45    | 333      | 14.8 |
|                                                  | PHF46    | 247      | 13.2 |
|                                                  | PHF47    | 111      | 14.8 |
|                                                  | PHF48    | 277      | 15.5 |
|                                                  | PHF49    | 259      | 15.5 |
|                                                  | PHF50    | 210      | 15.5 |
|                                                  | PHF51    | 233      | 10.5 |
|                                                  | PHF52    | 319      | 15.5 |
|                                                  | PHF53    | 158      | 15.5 |
|                                                  | PHF54    | 506      | 15.5 |
|                                                  | PHF55    | 131      | 20.5 |
|                                                  | PHF56    | 271      | 14.4 |
|                                                  | PHF57    | 148      | 22.7 |
|                                                  | RPL10    | 361      | 22.7 |
|                                                  | RPL11    | 245      | 22.7 |
|                                                  | RPL12    | 382      | 22.7 |
|                                                  | RPL13    | 399      | 22.7 |
|                                                  | RPL14    | 412      | 22.7 |
|                                                  | RPL15    | 713      | 10.4 |
|                                                  | RPL16    | 536      | 10.4 |
|                                                  | RPL17    | 619      | 10.4 |
|                                                  | RPL18    | 701      | 10.4 |
|                                                  | RPL19    | 283      | 17.0 |
|                                                  | RPL20    | 357      | 17.0 |
|                                                  | RPL21    | 157      | 17.0 |
|                                                  | RPL22    | 157      | 17.0 |
|                                                  | RPL23    | 200      | 17.0 |
|                                                  | RPL24    | 169      | 17.0 |
|                                                  | RPL25    | 207      | 17.0 |
|                                                  | RPL26    | 255      | 25.4 |
|                                                  | RPL27    | 152      | 25.4 |
|                                                  | RPL28    | 152      | 25.4 |
DTAVATQSLQAEYDAIR
JATSDLROVESVYQQQR
JATSDLROVESVYQQQR
YVR
LQOUMLSQVVTQVLK

Familin-3
PLNS3
QOQSYFYR
QGGFSKEDFPEQSEVR
SEEVADNMLFTKELSR
SVVTQNGVSMQR
VLADKKEETSR
VSGAVGEMNKDSKDVATQSLQ
AVDAETR

Presenilin-1
PRDX1
PRDX1
VCGQEFSDK
QFAYRSEEESEKVR

Polyubiquitin-binding protein 2
PABPN1
PABP2
TLSALDESLFR
VELEQDFMPK

Pre-mRNA-splicing regulator WTAP
WTAP
PL2D
QQLAQFOQQQQQQASAPSTSR
CQOSSQVVVR
PLSLPEVK
QGGRLFYDPQFLR
HFDIDSEYAGVR
NNDDFYTVQEGYVR
SICEGAVYQR
AHRVLCAELR
AFSEEVER
GEEFVAGP
FDAMIFTLR
ITSOFFEDLYK
LVKPMQPSLYVTEAVNK
RFDAMIFTLR
LLFVMQFPK

Probable sense carboxypeptidase CPVL
CPVL
CPVL
GGILRFPYDPQFLR
HFDIDSEYAGVR
NNDDFYTVQEGYVR
SICEGAVYQR
AHRVLCAELR
AFSEEVER
GEEFVAGP
FDAMIFTLR
ITSOFFEDLYK
LVKPMQPSLYVTEAVNK
RFDAMIFTLR
LLFVMQFPK

Proliferation-associated protein 204
PA204
PA204
QCGQRALEYR
NALPSVGEGK
EPPISQAK
EYVSMQQR
FFAFQVGCK
FTPQSKDLK
FAPYSQGCHK
OYFIFLRFK
QTVMLERK

Professional ubiquitin receptor ADRM1
ADRM1
ADRM1
QGQIRQDESQDKR
LRFVMQFPK
ATLYTAIEDR

Protein arginine N-methyltransferase 1
PRMT1
ANM1
QGSTACUR
TOEFEFGTCDFFAEK
ALAPVTHGAFLGISEYVK
EPFGQAGVK
EYVSMQQR
FFAFQVGCK
FTPQSKDLK
FAPYSQGCHK
OYFIFLRFK
QTVMLERK

Protein BLOC8S-TSNDC5
TSNDC5
QBMUY0
LAEVCTAER
LAEVCTAER
IKCAVQCHYELCNGQVR
TLPATVEELK
TLPATVEELK
VECTCHADSVCQAGQYR
VECTQCHYELCNGQVR
VYVAQTVCAHDESEVQCAQGV

Protein deoxi-isoamor family A, member 3, isoform CRA_b
PDE3A
PDE3A1
GPEA32
GQH2MCAK
MDATANDFPYVVR
QAGAPSVDETTFESFK
NPEDUATKL

Protein deoxi-isoamor family A6
PDEA6
PDEA6
B5M0Q5

Protein target transferase type-1, subunit alpha
FNTA
FNTA
LVPHNESAANYK
OLYQVSK
YIVSTINTGNYR

S17
| Protein NDRG1 | NDRG1 | NDRG1 | NDRG1 |
|--------------|-------|-------|-------|
| Sequence 1   | S18   | S18   | S18   |
| Sequence 2   | S18   | S18   | S18   |
| Sequence 3   | S18   | S18   | S18   |
| Sequence 4   | S18   | S18   | S18   |
| Sequence 5   | S18   | S18   | S18   |
| Sequence 6   | S18   | S18   | S18   |
| Sequence 7   | S18   | S18   | S18   |
| Sequence 8   | S18   | S18   | S18   |
| Sequence 9   | S18   | S18   | S18   |
| Sequence 10  | S18   | S18   | S18   |
| Sequence 11  | S18   | S18   | S18   |
| Sequence 12  | S18   | S18   | S18   |
| Sequence 13  | S18   | S18   | S18   |
| Sequence 14  | S18   | S18   | S18   |
| Sequence 15  | S18   | S18   | S18   |
| Sequence 16  | S18   | S18   | S18   |

S18
| Protein                      | Accession | EC Number | HMPO | Oligomeric State | Molecular Weight | Protein Name          | PubMed Reference |
|------------------------------|-----------|-----------|------|------------------|------------------|----------------------|------------------|
| Tubulin alpha-4 C chain     | TUBA4C    |           |      |                  |                  |                      |                  |
| Tubulin alpha-4 A chain     | TUBA4A    |           |      |                  |                  |                      |                  |
| Tubulin beta chain          | TUBB      |           |      |                  |                  |                      |                  |
| Tubulin beta-2 B chain      | TUBB2B    |           |      |                  |                  |                      |                  |
| Tubulin beta-4 A chain      | TUBB4A    |           |      |                  |                  |                      |                  |
| Tubulin beta-5 chain        | TUBB5     |           |      |                  |                  |                      |                  |
| Vimentin                     | VIM       |           |      |                  |                  |                      |                  |
| Vimentin                     | VIME      |           |      |                  |                  |                      |                  |

**WD repeat-containing protein 5**

| Accession | Molecular Weight | Protein Name | PubMed Reference |
|-----------|------------------|--------------|------------------|
| WDR5      |                  |              |                  |
| WDR5.5    |                  |              |                  |

**S19**
**Figure S13.** Confocal microscopy images of 1 in HeLa cells maintained at pH = 4, 5, 6, and 7, respectively. The cells were pretreated with media containing 1 (5.0 µM) for 5 h at 37 °C. The media were replaced with modified Krebs-Ringer buffer (5 mM NaCl, 125 mM KCl, 1 mM Na₃PO₄, 1 mM MgSO₄, 20 mM NaOAc, 10 mM HEPES) at various pH values (4-7) in the presence of nigericin and monensin (5.0 µM each). After 30 min, the images were then recorded using an excitation wavelength of 488 nm and monitoring over the 500–640 nm spectral region. Note that the fluorescent staining of the nucleus in the acidic pH might be due to an excess of 1 (unlabeled) in the media.

**Figure S14.** (a) Fluorescence spectra of MitoTracker Red (MTR) (0.1 µM) recorded at different pH values (5.0, 6.0, 7.0, 7.5, and 8.0). (b) Plot of fluorescence intensity (FI) at 634 nm vs pH. All data were recorded using an excitation wavelength at 581 nm.
Figure S15. (a) Confocal microscopy images of HeLa cells treated with probe 1 (5.0 μM) and MitoTracker Red (MTR) (0.1 μM). (b) Plot of fluorescence intensity (FI) of MTR vs number of laser irradiation events. The fluorescence intensities are obtained from the green boxed area in the microscopy image shown in (a). The images for 1 and MTR were recorded using an excitation wavelength at 488 nm, and monitoring over the 500–550 nm and 680–750 nm spectral regions, respectively.

Figure S16. Confocal microscopy images of HeLa cells treated with probe 1 (5.0 μM) and MitoTracker Red (MTR) (0.1 μM). The images for 1 and MTR were recorded using excitation wavelengths of 488 nm (one-photon laser source: OP) and 750 nm (two-photon laser source: TP), and monitoring over the 500–550 nm and 680–730 nm spectral regions, respectively.
Figure S17. Pseudo color images produced from confocal images of (a) intact and (b) nutrient-deprived HeLa cells treated with probe 1 (5.0 μM) and MitoTracker Red (MTR) (0.1 μM). Color strip represents the pseudo color change with pH. All images were recorded using an excitation wavelength of 488 nm, and monitoring over the 500–550 nm and 680–750 nm spectral regions.
Figure S18. Time course microscopy images of HeLa cells treated with probe 1 (5.0 μM) and LysoTracker Red (LTR) (1.0 μM) in growth medium (for intact cell). Images for 1 and LTR were collected at 15 sec intervals from baseline. Panels show the merged images of 1 and LTR. All images were recorded using an excitation wavelength of 488 nm, and monitoring over the 510–550 nm and 570–660 nm spectral regions.
**Figure S19.** Time course microscopy images of HeLa cells treated with probe 1 (5.0 µM) and LysoTracker Red (LTR) (1.0 µM) in serum-free KRH (Krebs-Ringer-HEPES) buffer containing glucagon (1.0 µM) and pepstatin A (7.5 µM) (for starved cell). Images for 1 and LTR were collected at 15 sec intervals from baseline. Panels show the merged images of 1 and LTR. All images were recorded using an excitation wavelength of 488 nm, and monitoring over the 510–550 nm and 570–660 nm spectral regions.
Figure S20. Time course microscopy images of HeLa cells treated with probe 1 (5.0 µM) and LysoTracker Red (LTR) (1.0 µM) in serum-free KRH (Krebs-Ringer-HEPES) buffer containing glucagon (1.0 µM) and pepstatin A (7.5 µM) (for starved cell). Images were recorded at time points consisting of 75, 165, and 345 sec from baseline. The white boxed areas in the image at t = 0 was then enlarged. Arrows: dots represent mitochondrial fusion with lysosomes. All images were recorded using an excitation wavelength of 488 nm, and monitoring over the 510–550 nm and 570–660 nm spectral regions.
11. $^1$H-NMR, $^{13}$C-NMR and ESI-MS spectra

Figure S21. $^1$H-NMR spectrum of 1 in CDCl$_3$.

Figure S22. $^{13}$C-NMR spectrum of 1 in CDCl$_3$.
Figure S23. HRESI-MS spectrum of 1.

Figure S24. $^1$H-NMR spectrum of 2 in CDCl$_3$. 
Figure S25. $^{13}$C-NMR spectrum of 2 in CDCl$_3$.

Figure S26. HRESI-MS spectrum of 2.
Figure S27. $^1$H-NMR spectrum of 3 in CDCl$_3$.

Figure S28. $^{13}$C-NMR spectrum of 3 in CDCl$_3$. 

$\text{C}_{19}\text{H}_{18}\text{BrNO}_4$
Mol. Wt.: 404.25

3
Figure S29. HRESI-MS spectrum of 3.

Figure S30. $^1$H-NMR spectrum of 4 in CDCl$_3$. 

C$_{23}$H$_{27}$N$_3$O$_4$
Mol. Wt.: 409.48
Figure S31. $^{13}$C-NMR spectrum of 4 in CDCl$_3$.

Figure S32. HRESI-MS spectrum of 4.
Figure S33. $^1$H-NMR spectrum of 5 in CDCl$_3$.

Figure S34. $^{13}$C-NMR spectrum of 5 in CDCl$_3$.
**Figure S35.** HRESI-MS spectrum of 5.

**Figure S36.** $^1$H-NMR spectrum of 6 in a mixture solvent of CDCl$_3$ and CD$_3$OD.
Figure S37. ESI-MS spectrum of 6.

Figure S38. $^1$H-NMR spectrum of 9 in CDCl$_3$/CD$_3$OD.
Figure S39. $^{13}$C-NMR spectrum of 9 in CDCl$_3$.

Figure S40. HRESI-MS spectrum of 9.
Figure S41. $^1$H-NMR spectrum of 10 in CDCl$_3$.

Figure S42. $^{13}$C-NMR spectrum of 10 in CDCl$_3$. 
Figure S43. HRESI-MS spectrum of 10.

Figure S44. $^1$H-NMR spectrum of 11 in CDCl$_3$. 
Figure S45. $^{13}$C-NMR spectrum of 11 in CDCl$_3$.

Figure S46. HRESI-MS spectrum of 11.
References

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