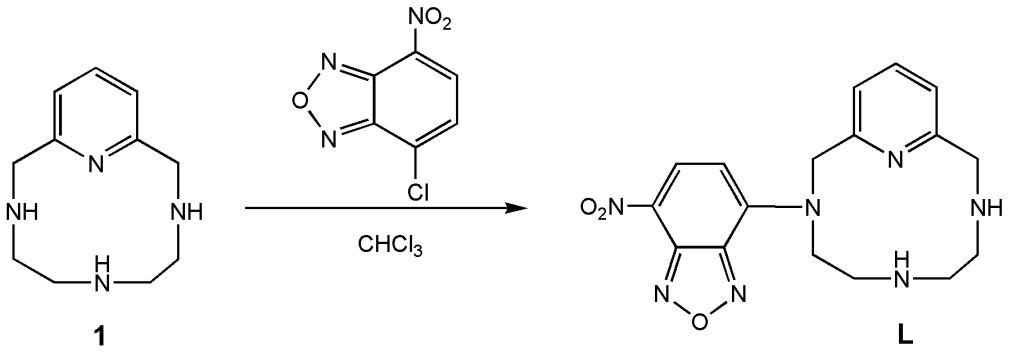
Experimental

General: All the starting materials were of reagent quality and were obtained from commercial sources without further purification. 3-(4,5-dimethyl-2-thiazoly)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Sigma. Hoechst 33358, Mitotracker Red, BODIBY TR ceramide, and DND-99 were bought from Keygen. All the emission spectra were obtained using a PerkinElmer LS 55 fluorescence spectrometer. The electrospray ionization mass spectra were determined by a LCQ Fleet ThermoFisher mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra in $CDCl_3$ were measured on a Bruker DRX-500 spectrometer at 25±1°C. The pH values of sample solutions were monitored by a PHS-3 system. Confocal fluorescence and brightfield imaging were performed with a Zeiss LSM 710 microscope. 1,4,7-Tritosyl-1,4,7-triazaheptane was prepared according to a reported procedure[1] in 64% yield. 2,6-Bis(bromomethyl)pyridine and the mother ring 3,6,9,15-tetraazabicyclo[9.3.1] pentadeca-1(15),11,13-triene (**1**) were synthesized following the procedure described by references[2,3] in the yields of 49% and 65%, respectively.

Synthesis of probe L:



4-chloro-7-nitrobenzofurazan (NBD-Cl) (199 mg, 1 mmol) in 50 mL of $CHCl_3$ was added to a 200 mL $CHCl_3$ solution of **1** (618 mg, 3 mmol) and Et_3N (102 mg, 1 mmol). The resulting mixture was then heated at 60°C and stirred for 4 h under N_2 ambient. After being cooled to room temperature, the obtained mixture was washed with 1 M NaOH solution (20 mL×3) and then with redistilled water (20 mL×3). The organic layer was dried over anhydrous sodium sulfate overnight and evaporated to give the solid crude product. The residue was purified over column chromatography and eluted with a gradient of chloroform to methanol (10:1, v/v), yielding the product **L** as a dark red solid (155 mg, 42%). Mass: ESI-MS (CH_3CN), m/z (%): 370.33(100) [MH^+]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (d, J = 9.1 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 6.47 (s, 1H), 5.32 (d, J = 18.7 Hz, 2H), 4.57 (s, 2H), 3.92 (s, 2H), 3.06 (s, 2H), 2.80 (s, 2H), 2.68 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δC 160.02, 154.61, 146.23, 144.78, 137.79, 135.28, 129.78, 127.35, 121.13, 120.26, 103.25, 59.02, 55.07, 52.02, 48.52, 47.75, 46.00 ppm.

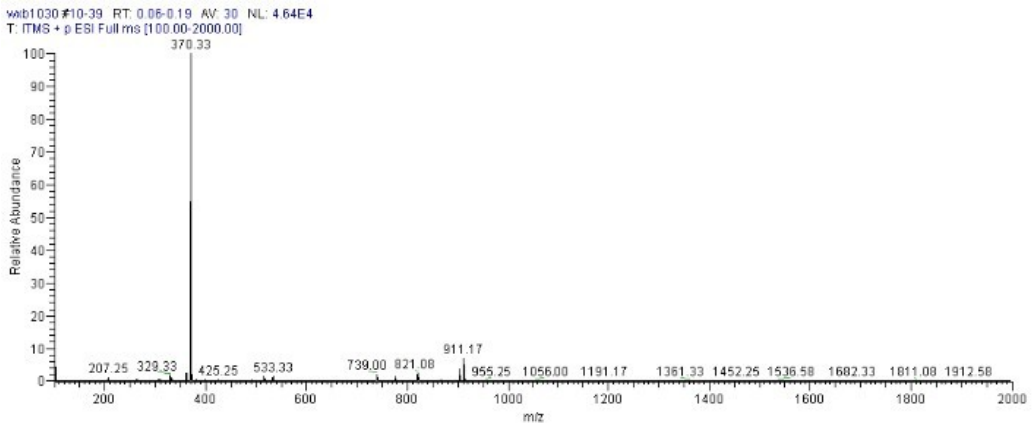


Fig. S1 ESI-MS spectrum of **L** in CH_3CN.

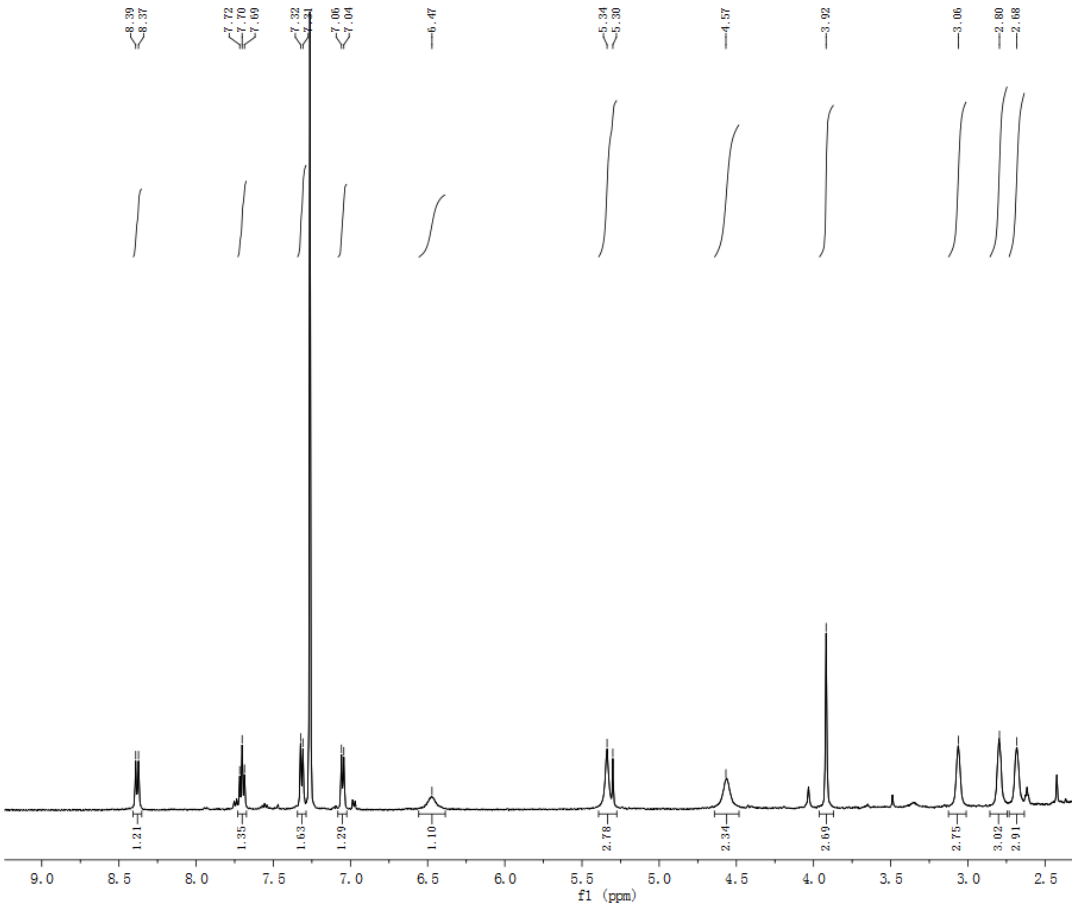


Fig. S2 ^1H NMR spectrum of **L** in $CDCl_3$ (500 MHz).

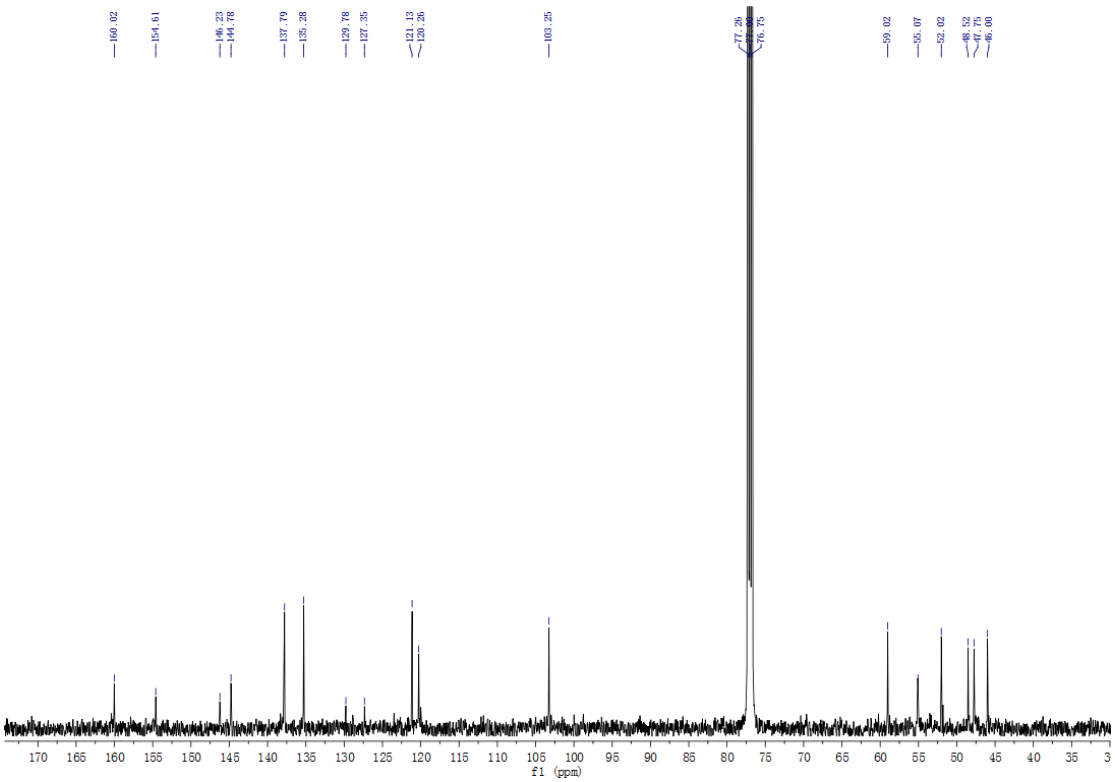


Fig. S3 ^{13}C NMR spectrum of **L** in $CDCl_3$ (126 MHz).

Cell culture:

Human cervix carcinoma HeLa cells were obtained from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China. Cells were grown in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) in a humidified atmosphere at 37 °C.

Cytotoxicity assay:

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were applied to test cell viability. Briefly, 2×10^4 HeLa cells were seeded onto 96-well plates (Corning, NY, USA) and incubated for 12 h. Then different concentrations of **L** were added to the wells and incubated for 24 h. Thereafter, 20 μL of MTT (0.5 $mg\cdot cm^{-3}$ final concentration) was added to each well. After incubation for 4 h, 150 μL of DMSO was added to the cultures to dissolve the crystals. The absorption values at 570 nm were monitored by an automatic enzyme-linked immunosorbent assay plate reader (Thermo Scientific, USA).

Cell imaging:

5×10^4 HeLa cells were plated in Confocal dish (Corning, NY, USA) and incubated for 12 h. For co-staining studies, cells were incubated with Hoechst 33358 (Invitrogen, final concentration 0.8 μM) or Mitotracker Red (Invitrogen, final concentration 25 nM) for 10 min or LysoTracker Red (Invitrogen, final concentration 50 nM) for 30 min at 37 °C before being stained with **L** (100 μM) for 30 min at 37 °C. Incubations with BODIPY TR ceramide (Invitrogen, final concentration 5 μM) were performed for 30 min at 4°C, followed by three wash steps with ice-cold HBBS/HEPES (200 mL) and an additional incubation for 30 min at 37 °C prior to being stained with **L** (100 μM). Fluorescence images were obtained with a laser scanning confocal fluorescence microscope (Zeiss LSM 710).

ICP-MS analysis:

5×10^4 HeLa cells were plated and treated with **L** and $CuCl_2$. Cells were collected and stored at -80 °C until further testing. 100 μL of concentrated nitric acid (HNO_3) was added to each sample and heated for 2 h at 90 °C. Samples were allowed to add 25 μL H_2O_2 for another 2 h at 90 °C. Then the samples were added 40 μL of concentrated hydrochloric acid (HCl) and digested overnight at room temperature and diluted to 1:25 with 1% HNO_3 prior to analysis. Analysis was carried out on a Varian UltraMass ICP-MS instrument that had been calibrated using 0, 10, 50, and 100 ppb of a certified multielement ICP-MS standard solutions (ICP-MS-CAl2-1, AccuStandard). A certified internal standard solution containing 100 ppb copper was also used as the internal control (ICPMS-IS-MIX1-1, AccuStandard).

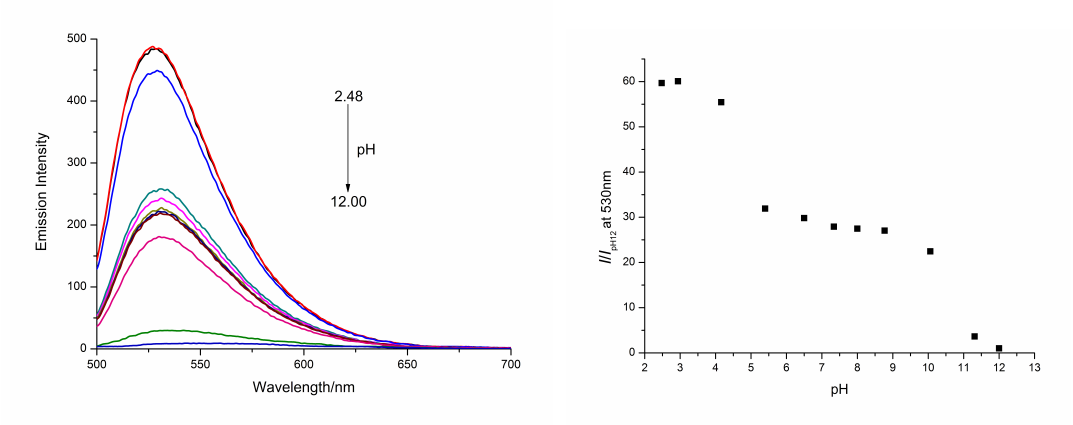


Fig. S4 Emission spectra of 20 μM **L** in water containing 0.15 M NaCl at different pH (left). λ_{ex} = 470 nm. Fluorescent pH titration profile of **L** according to $I/I_{pH12.00}$ at 530 nm (right).

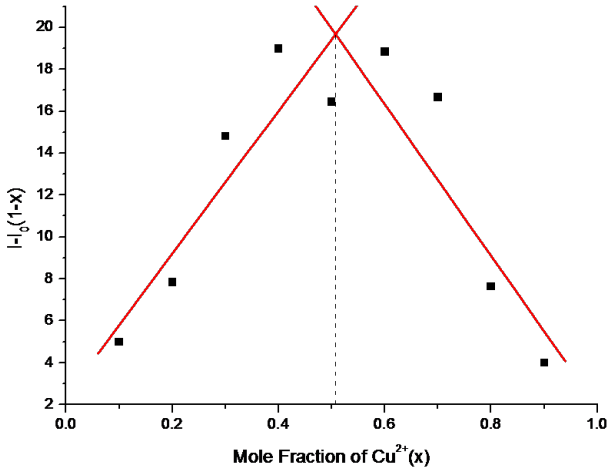


Fig. S5 Job's plot for the binding between **L** and Cu^{2+}. [**L**] + [Cu^{2+}] = 20 μM.

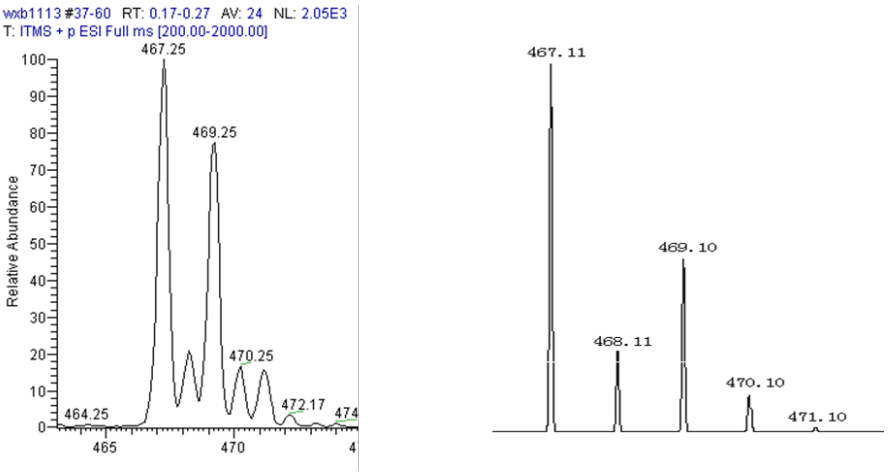


Fig. S6 Observed electrospray ionization (ESI) mass spectrum and calculated isotop patterns for **L**-Cu^{2+}.

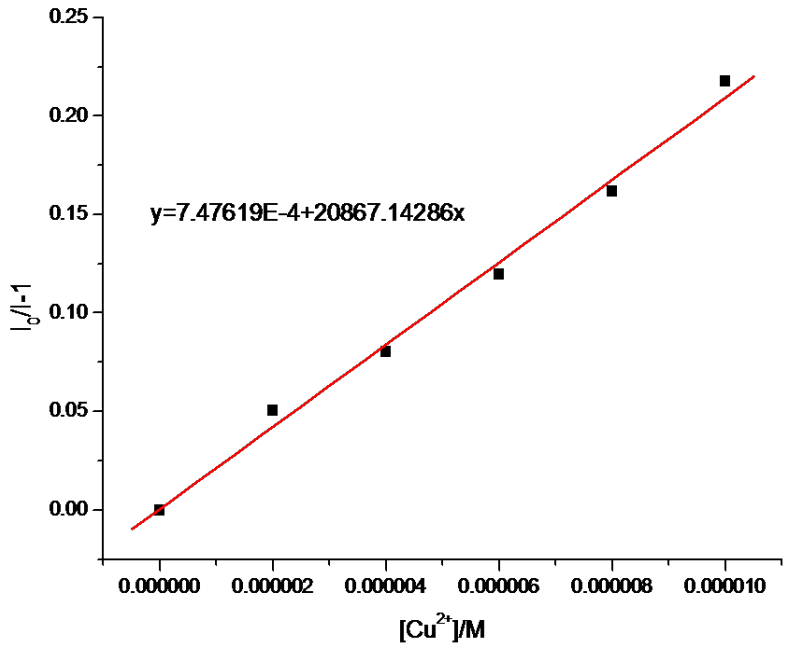


Fig. S7 Emission change of **L** induced by Cu^{2+} titration. The binding constant was obtained by linear fitting to the data, and the red line is the resulting fitting line.

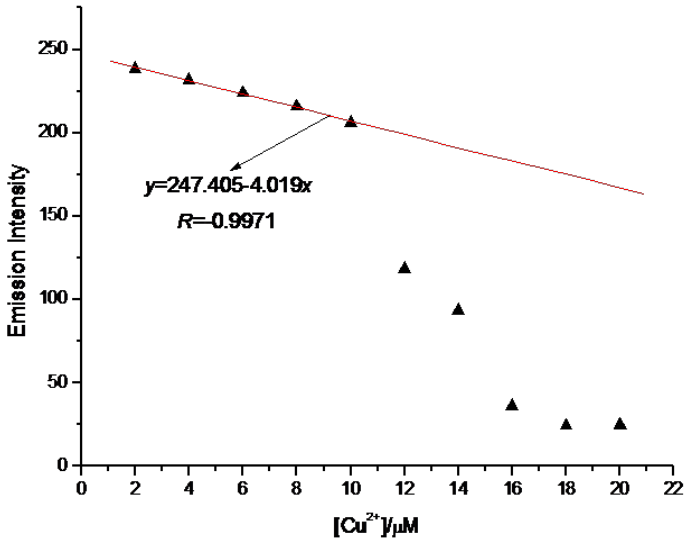


Fig. S8 A plot of emission intensity *vs* [Cu^{2+}], the standard deviation (SD) is 1.1297 and the calculated detection limit of **L**-Cu^{2+} is 8.4×10^{-7} M..

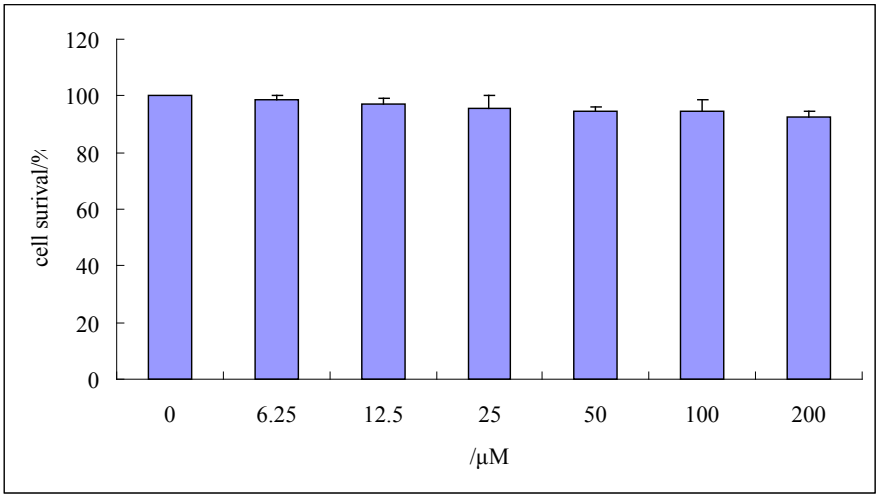


Fig. S9 MTT experiments of **L** performed with HeLa cells.

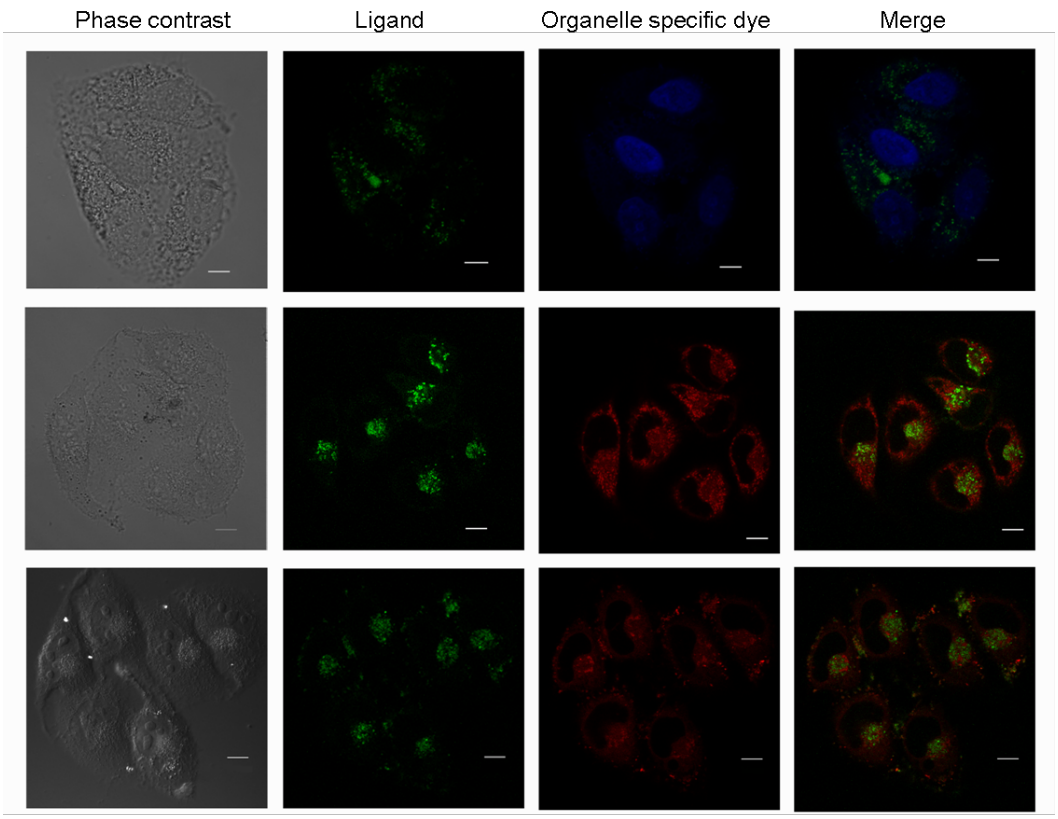


Fig. S10 Co-localization studies of **L** with organelle-specific dyes in HeLa cells. Cells were stained with nucleus maker Hoechst 33258 (0.8 μM) for 30 min at 37°C, or mitochondrial dye Mitotracker Red (25 nM) for 30 min at 37°C, or Golgi dye BODIPY TR ceramide (5 μM) for 30 min at 4°C, before being incubated with **L** (100 μM) for 30 min at 37°C. Nucleus staining (blue), Mitochondria or Golgi staining (red) and **L** localization (green). λ_{ex} = 488 nm; Scale bars: 10 μm.

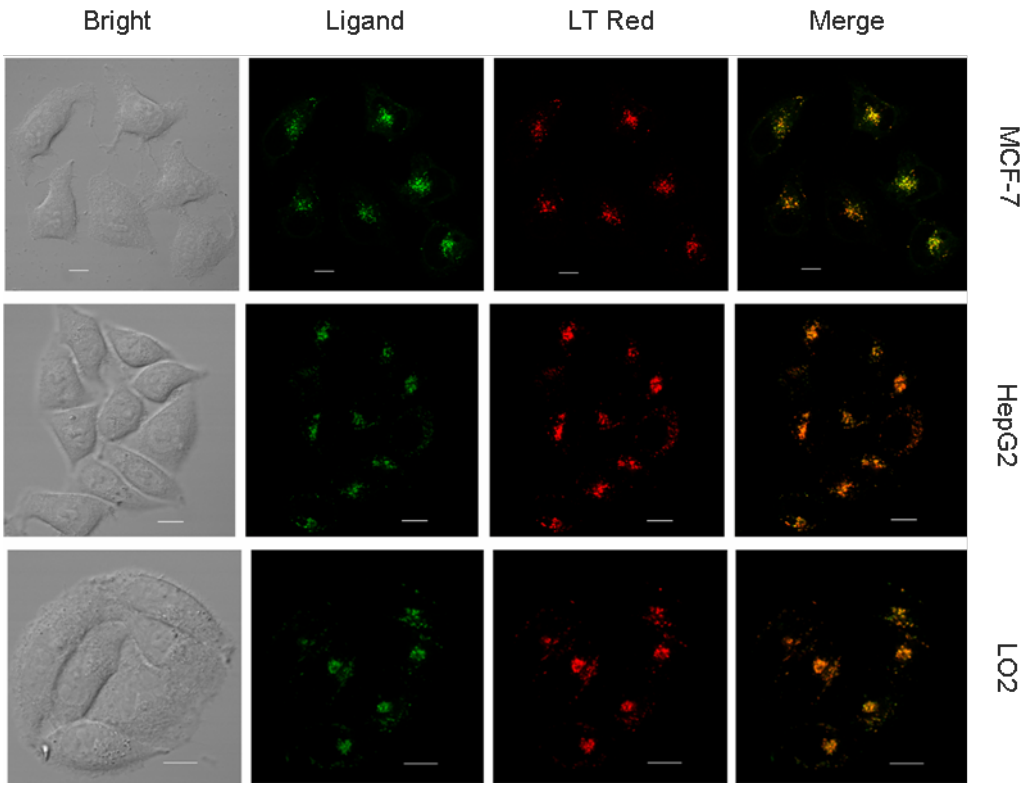


Fig. S11 Co-localization studies of **L** with LysoTracker Red in MCF-7, HepG2 and LO2 cells. Cells were stained with LysoTracker Red (50 nM) for 30 min at 37°C, before being incubated with **L** (100 μM) for 30 min at 37°C. λ_{ex} = 488 nm; Scale bars: 10 μm.

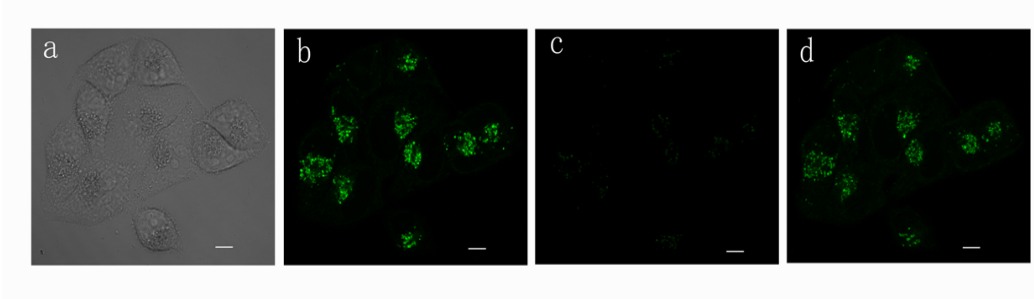


Fig. S12 Brightfield and fluorescence images of HeLa cells. (a) Brightfield image of cells, (b) cells stained with probe **L** (100 μM) for 30 min at 37°C, (c) then incubated with $CuCl_2$ (100 μM) for 30 min at 37°C after (a), (d) further incubated with TPEN (50 μM) for 10 min at 37°C. λ_{ex} = 488 nm; Scale bars: 10 μm.

References:

1 H.Koyama and T. Yoshino, *Bull. Chem. Soc. Jpn*., 1972, **45**, 481.
2 X. M. Zhang, *WO Pa*t., 97/13763, 1997.
3 G. E. Kiefer, J. Simon and J. R. Garlich, *WO Pat*., 94/26754,1994.