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A fluorescent flavonoid for lysosome detection in live cells under "wash free" conditions

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Supporting Information

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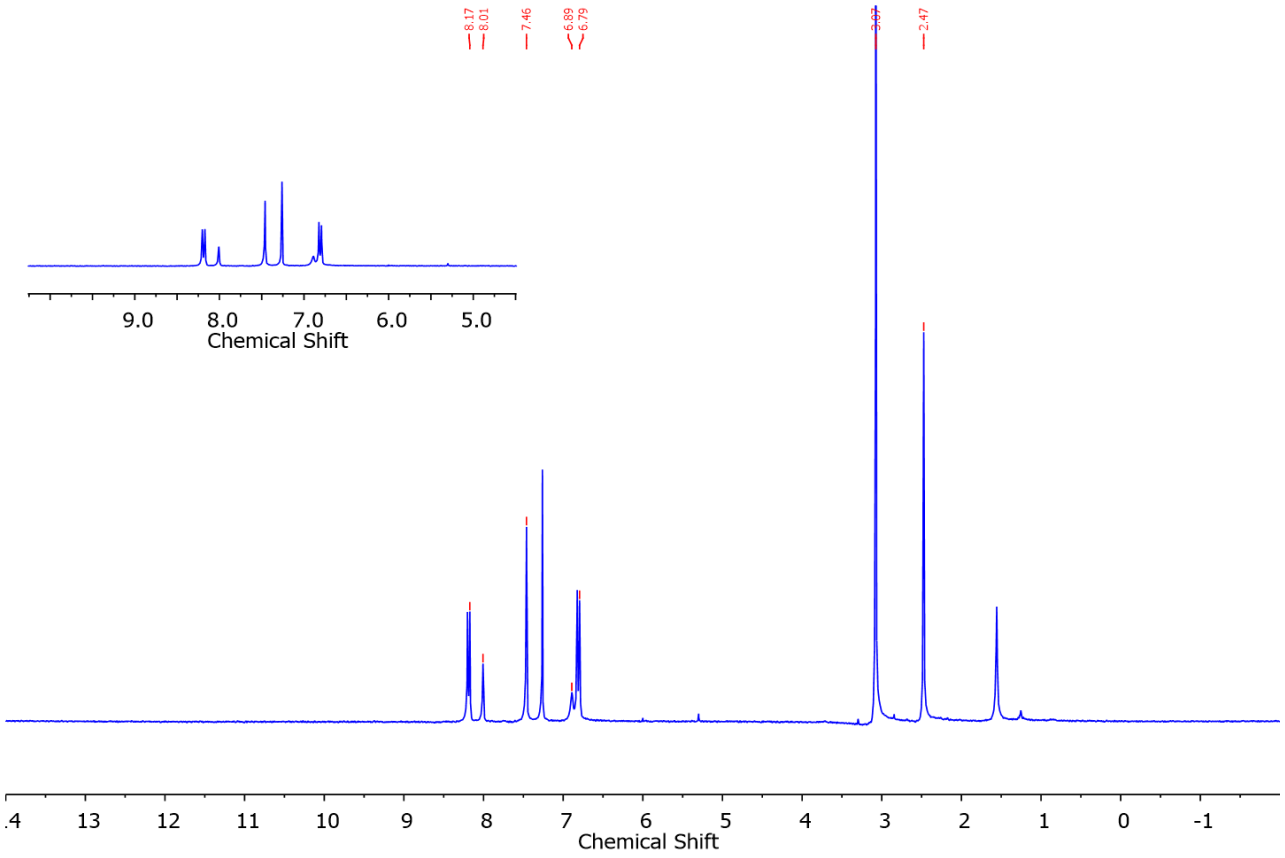


Figure S1.1 ^{1}H NMR spectra of **1** (300 MHz in $CDCl_3$)

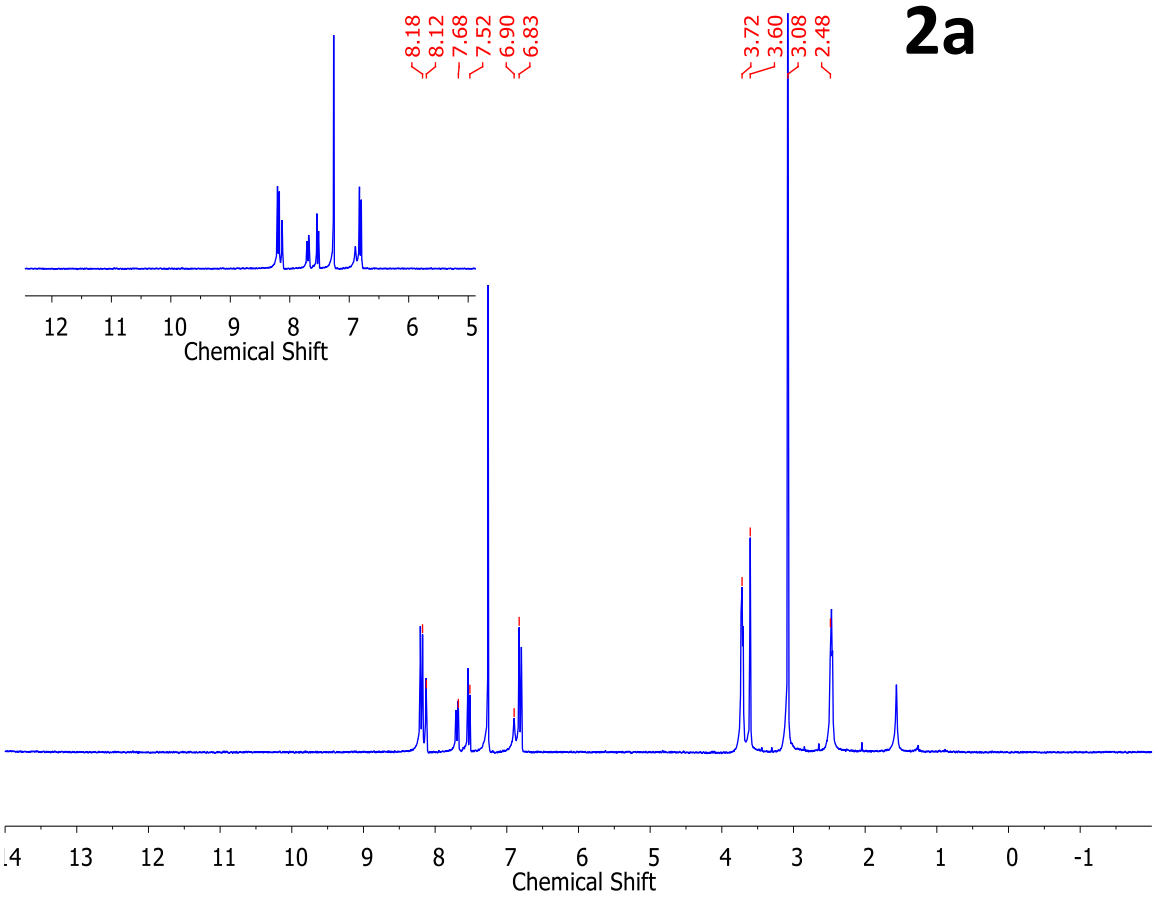


Figure S1.2 ^{1}H NMR spectra of **2a** (300 MHz in $CDCl_3$)

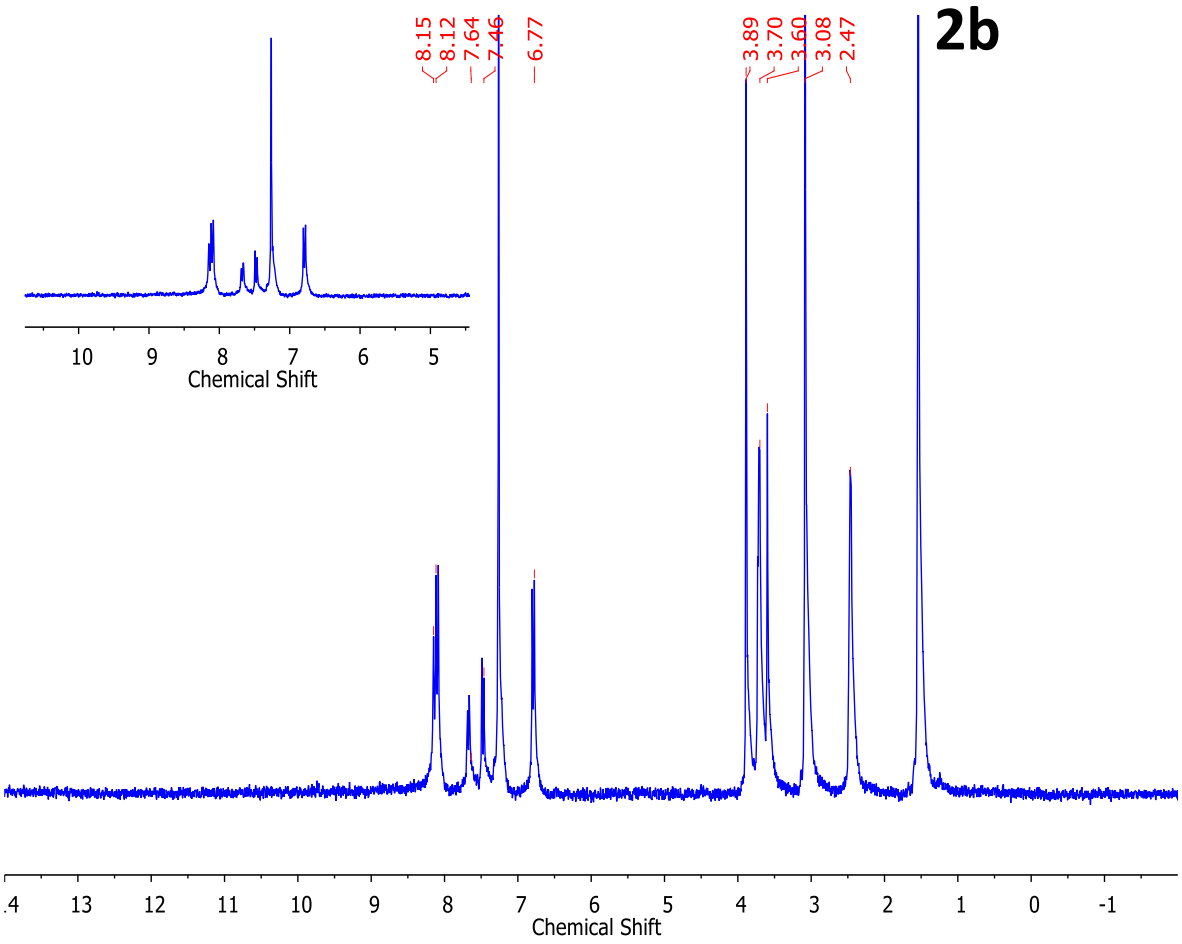


Figure S1.3 ^{1}H NMR spectra of **2b** (300 MHz in $CDCl_3$)

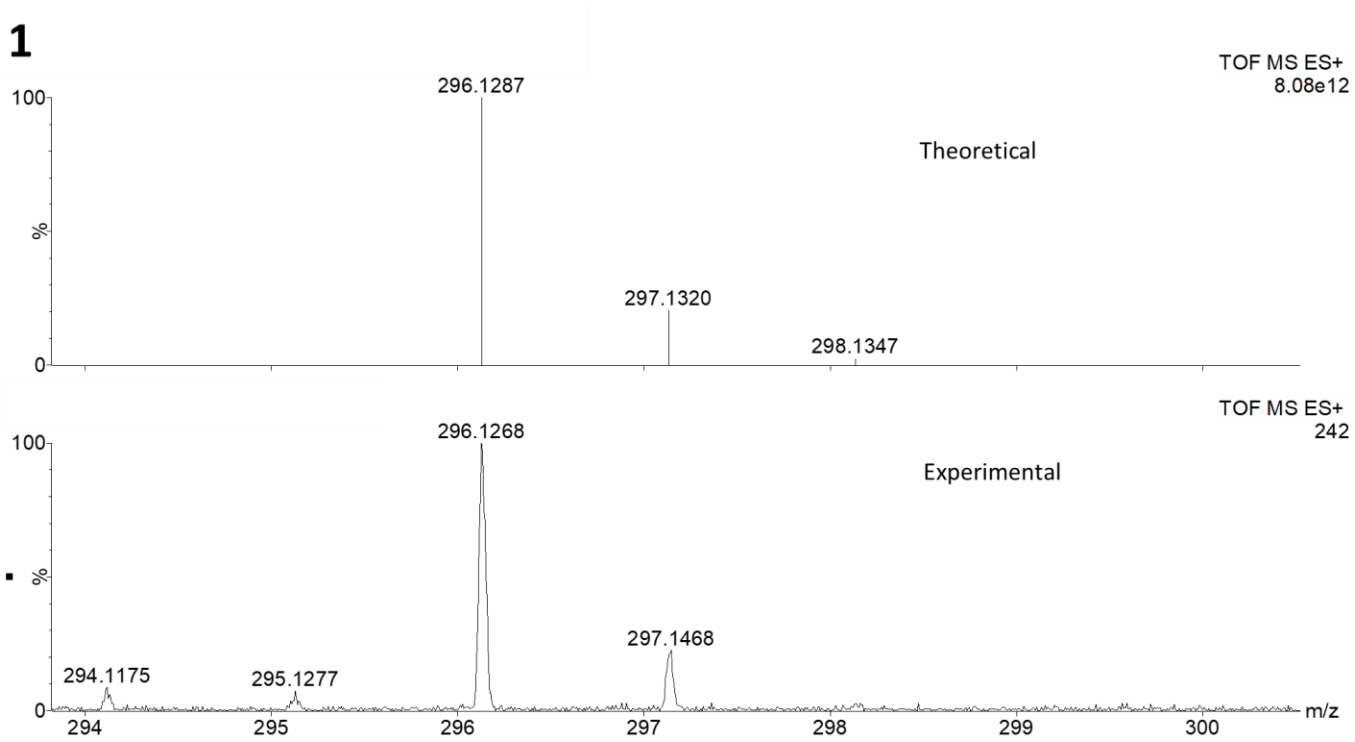


Figure S2.1 Mass spectra of **1**

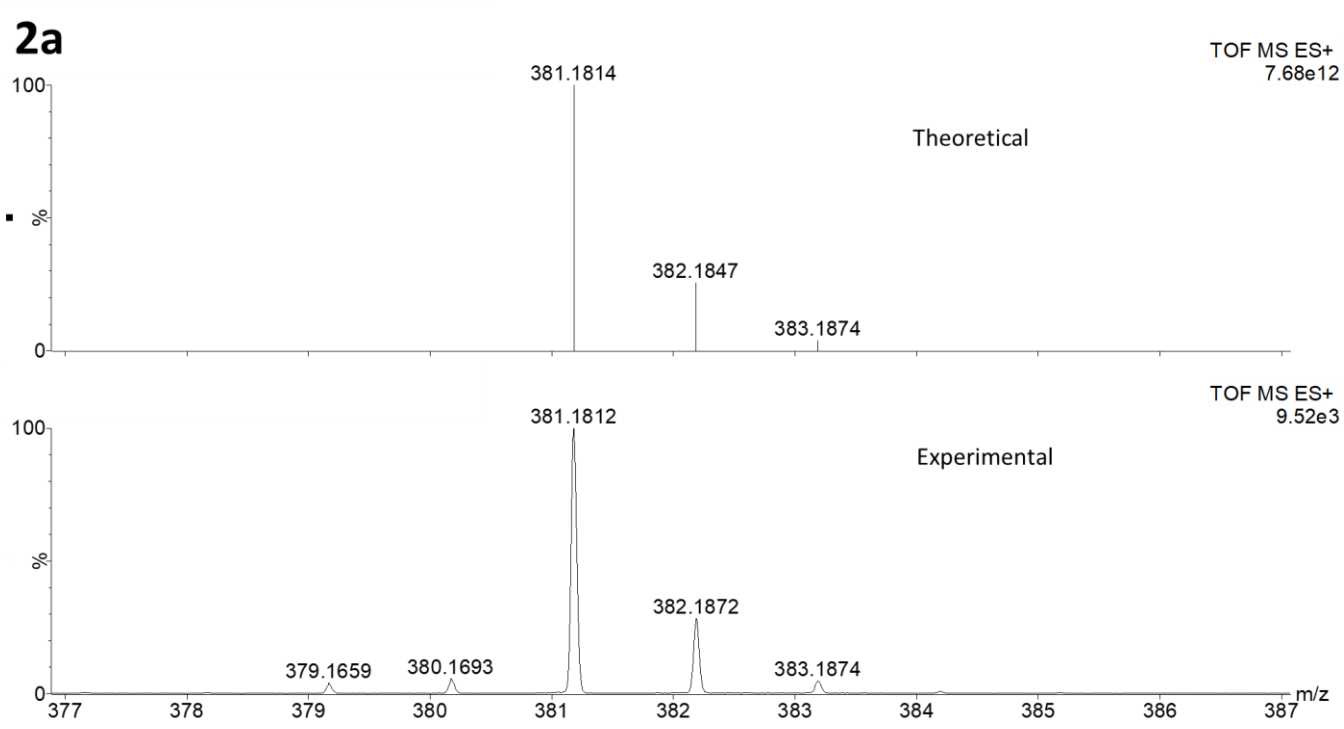


Figure S2.2 Mass spectra of **2a**

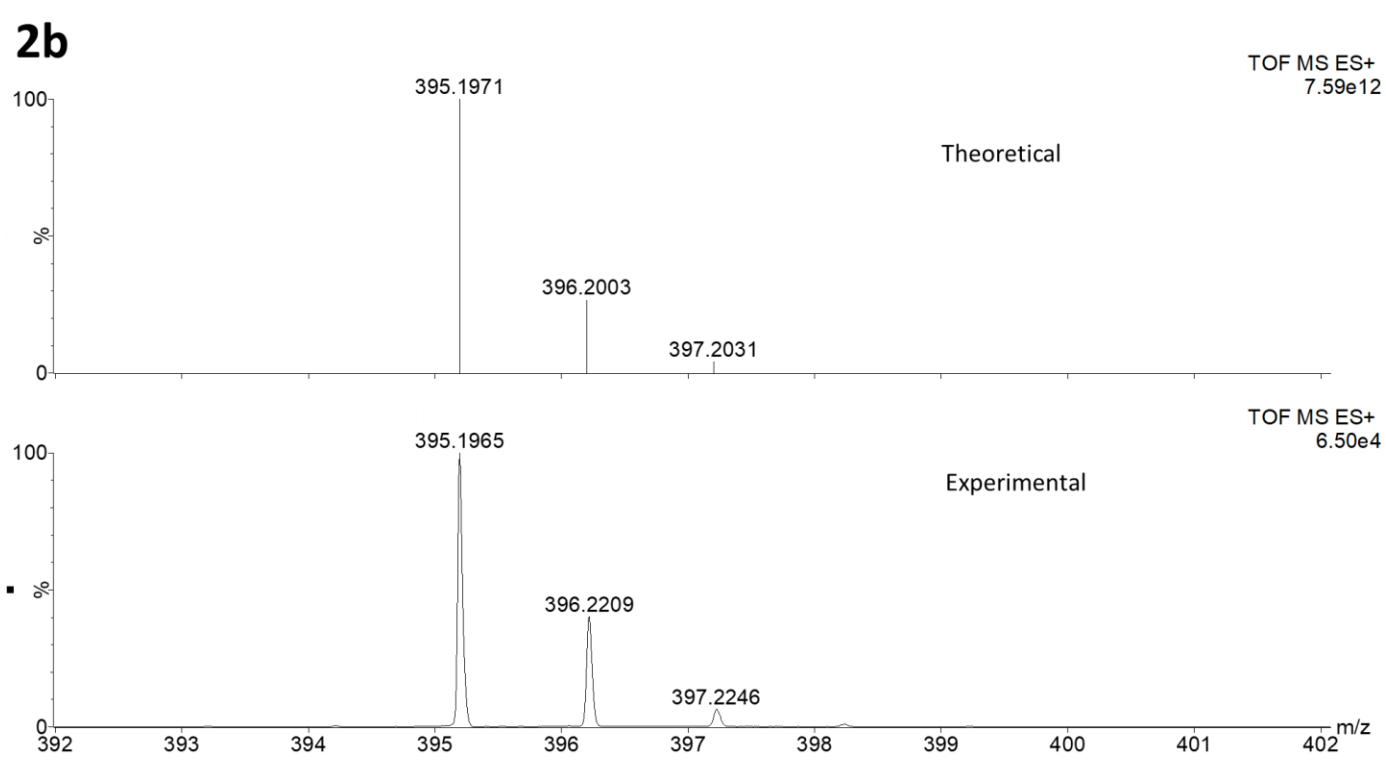


Figure S2.3 Mass spectra of **2b**

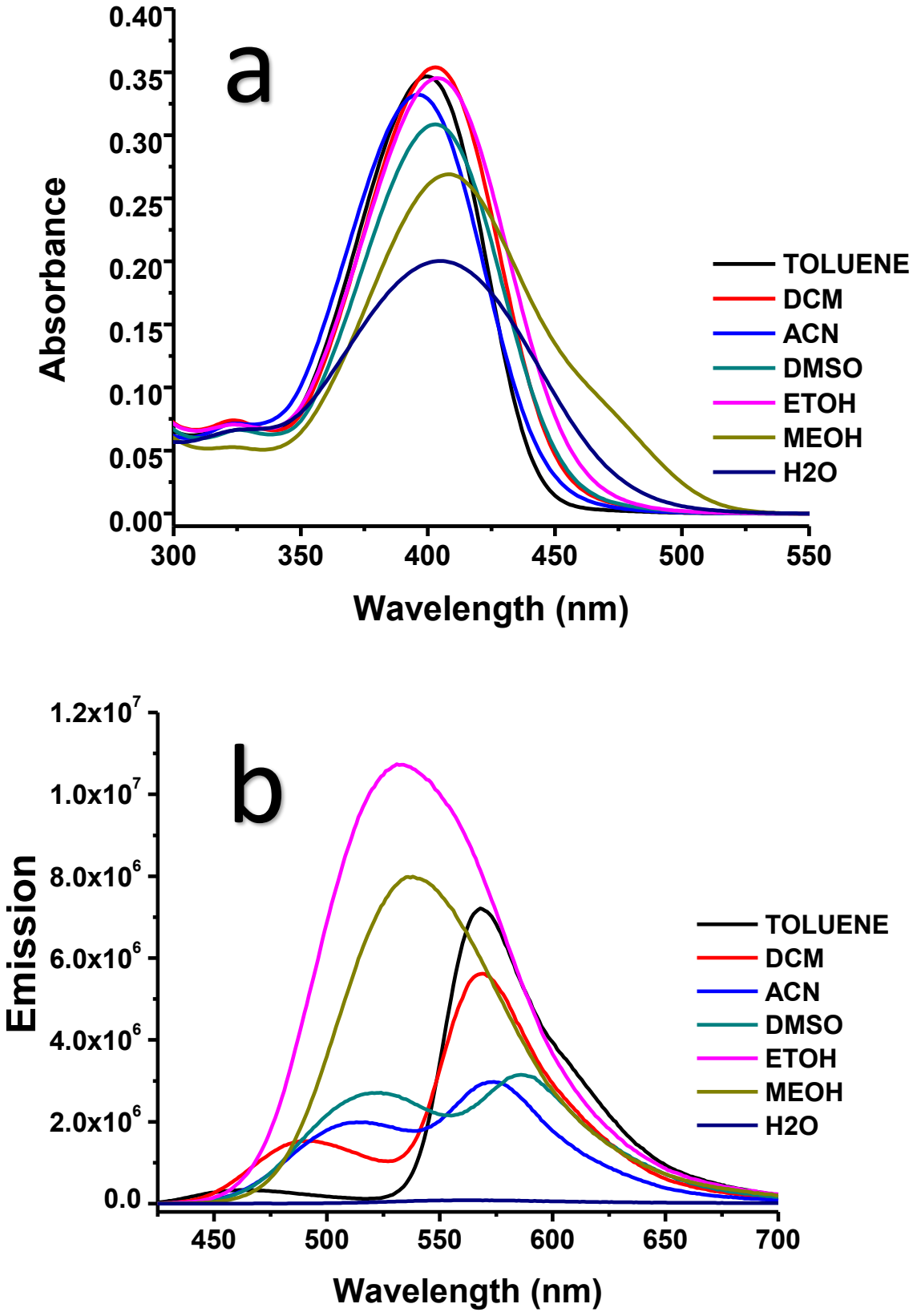


Figure S3.1 Absorbance (a) and fluorescence emission (b) spectra obtained for **2a** ($1x10^{-5}$ M) in different solvents at room temperature.

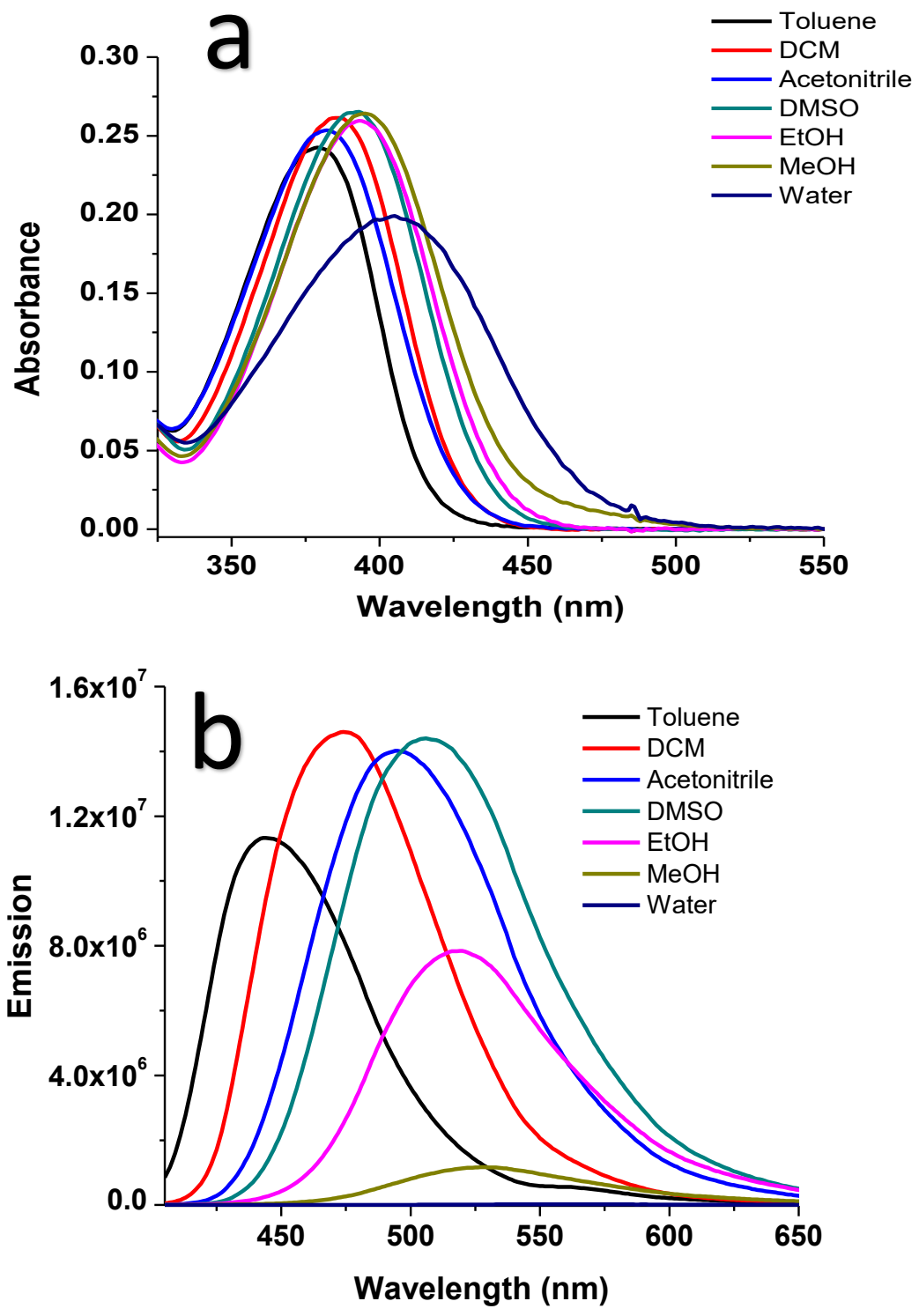


Figure S3.2 Absorbance (a) and fluorescence emission (b) spectra obtained for **2b** ($1x10^{-5}$ M) in different solvents at room temperature.

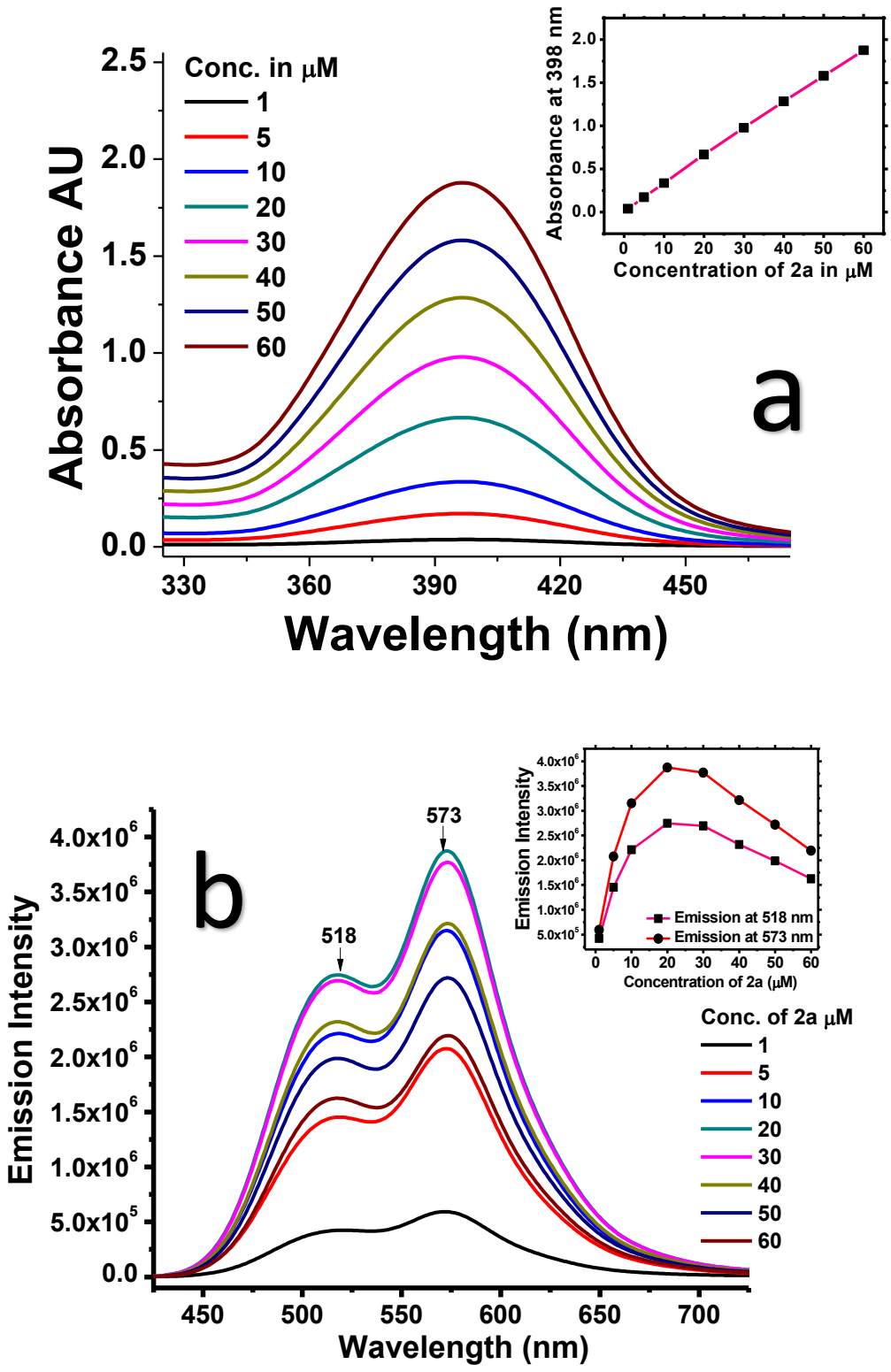


Figure S3.3 Absorbance (a) and fluorescence emission (b) spectra obtained for **2a** in DCM in different concentrations at room temperature.

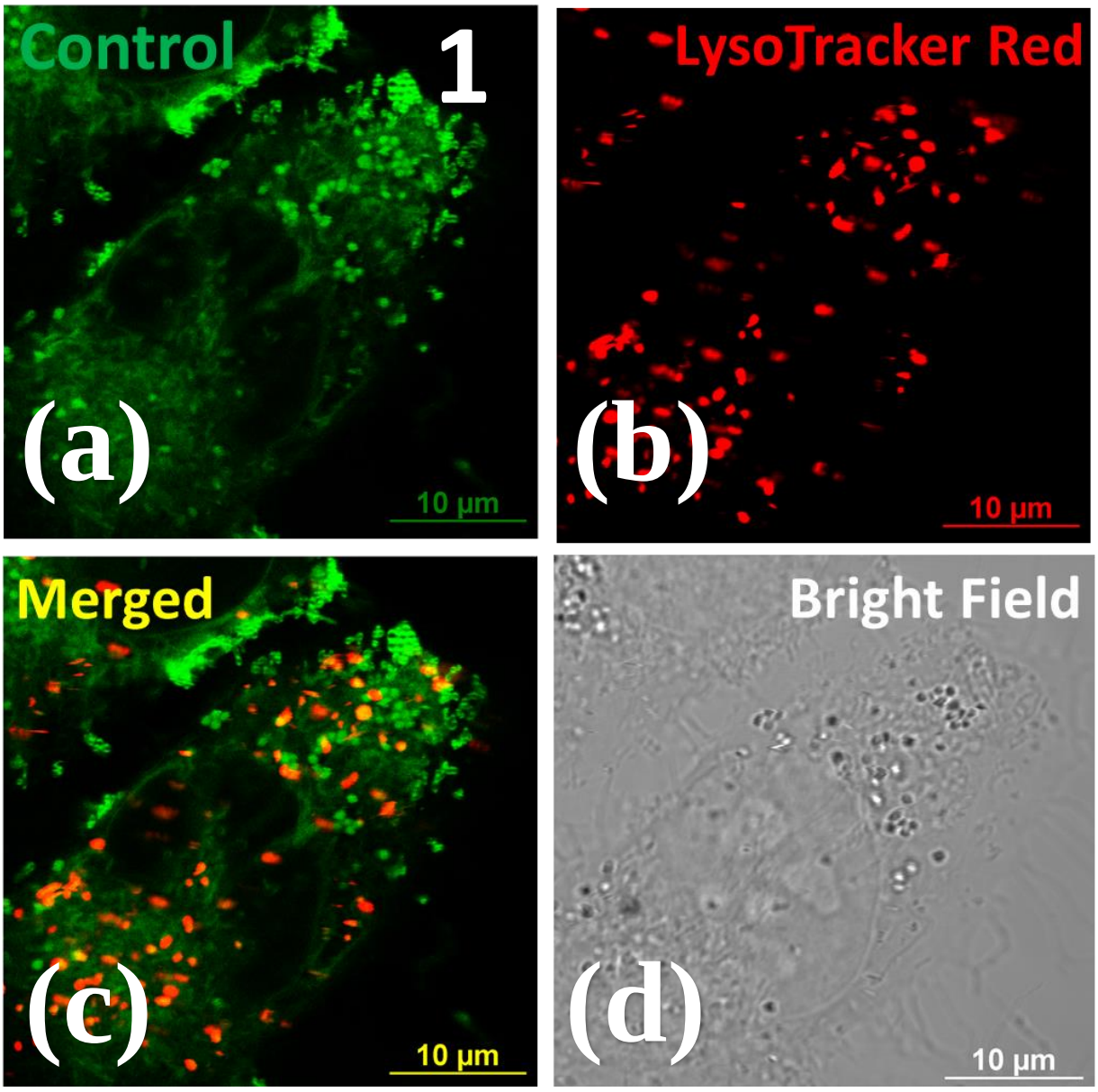


Figure S4 Fluorescent confocal microscopy images of MO3.13 cells (x100) treated with **1** (3 µM) for 30 minutes. Images show the fluorescence **1** (a), LysoTracker Red® (b), overlapped image (c), and bright field (d). Laser 405 nm and 561 nm were used for the green and red channels respectively.

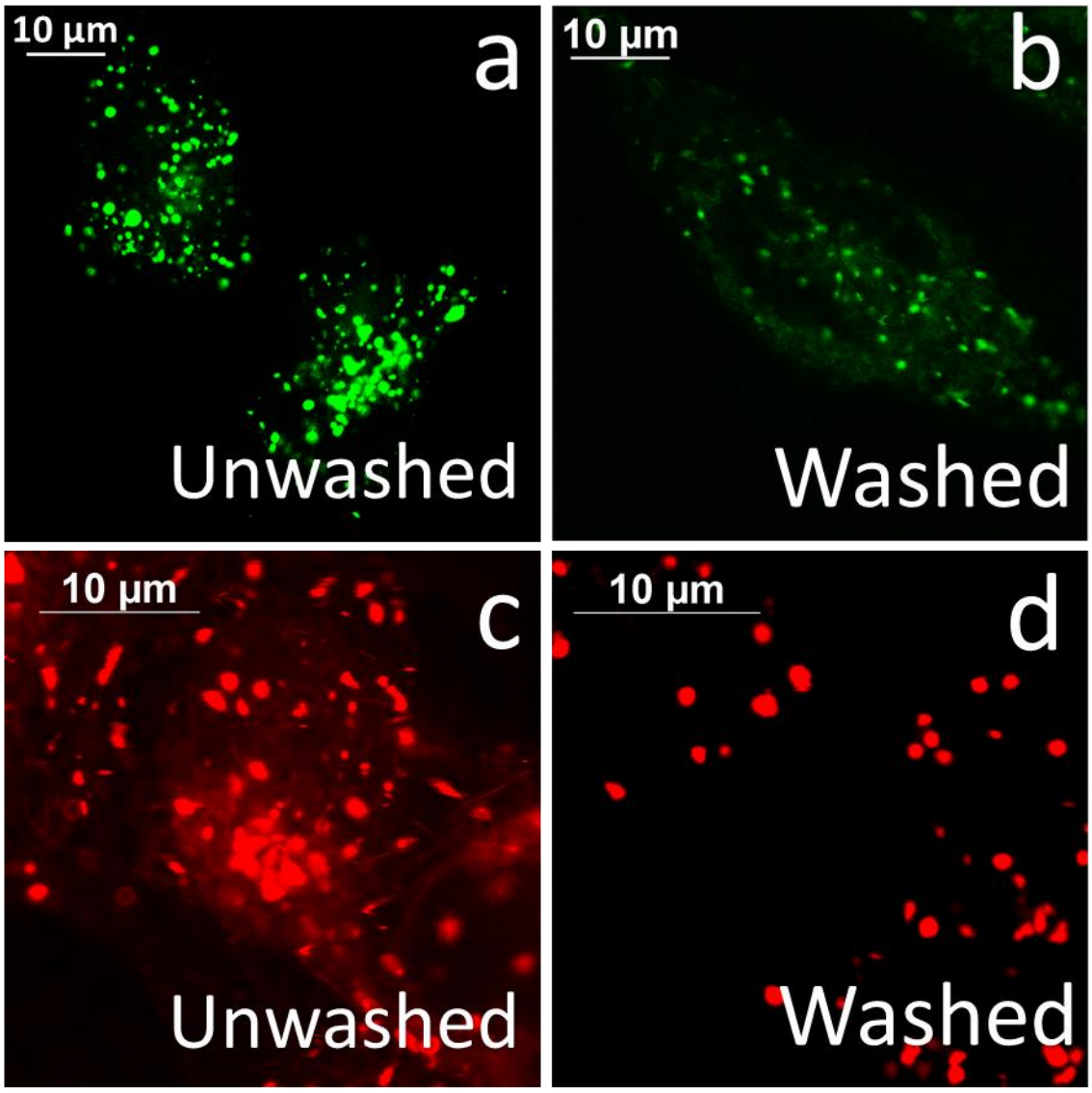


Figure S5. Confocal microscope images of NHLF cells stained with 200 nM Lysotracker Green® (a-b) and 75 nM Lysotracker Red® (c-d) for 30 minutes with digitally enlarged magnification (a-d). Lysotracker green® and red® were excited with lasers 488 nm and 561 nm, respectively.

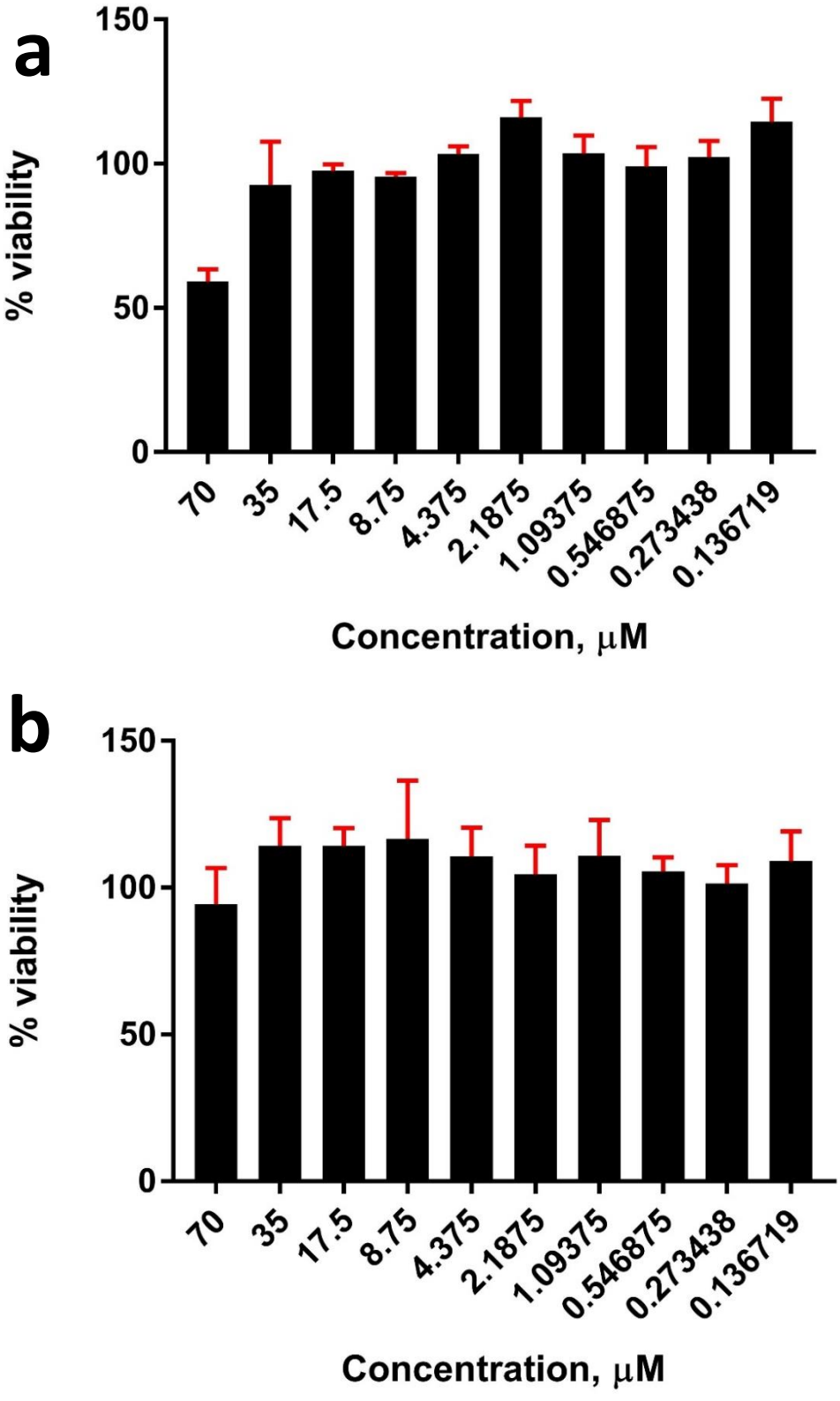


Figure S6 Cell viability results (bar-chart) obtained for **2a** (a) and **2b** (b) by MTT cell viability assay.

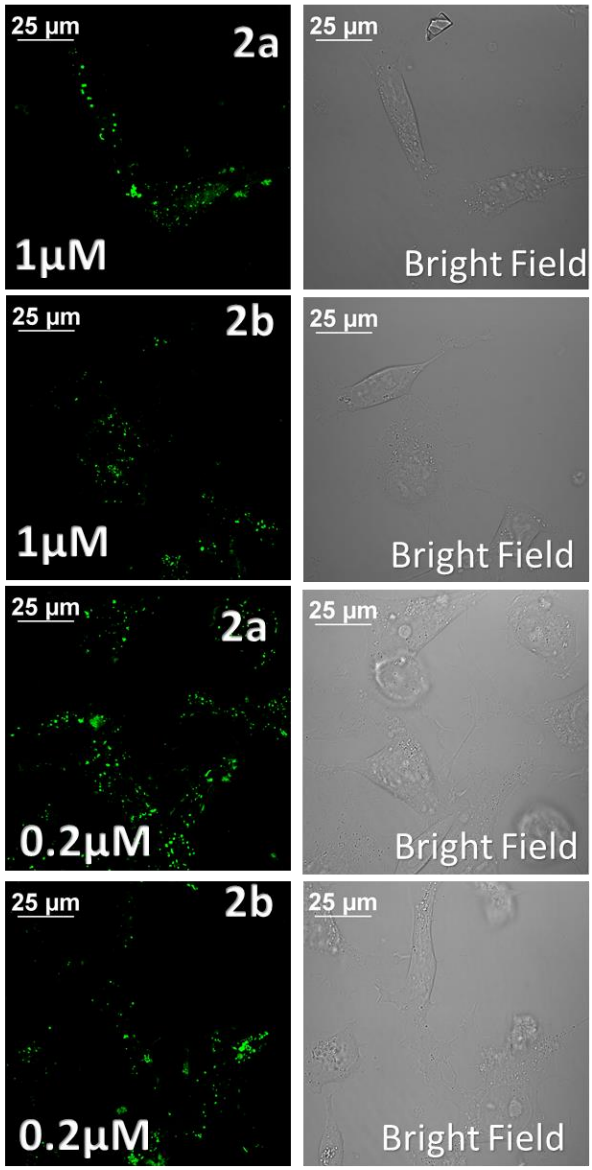


Figure S7. Confocal microscope images of MO3.13 cells stained with **2a** and **2b** at different concentrations for 30 minutes at 100x magnification. Compounds **2a** and **2b** were excited at 405nm. No post-staining washing was done for imaging. Filter region for emission is in the method and material section.

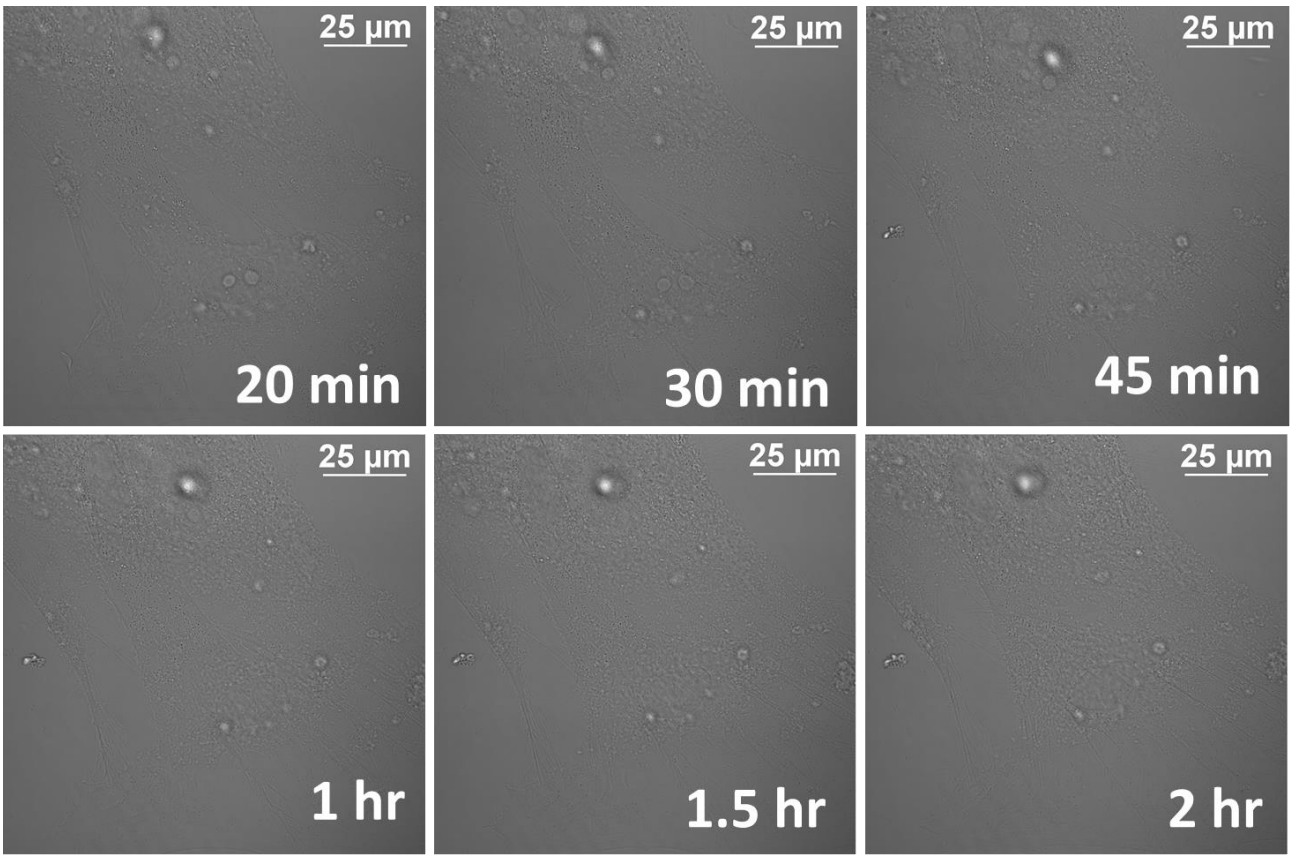


Figure S8. Bright field from Confocal microscope time study images of MO3.13 cells stained with 3 µM **2b** at 100x magnification. Compound was excited with 405 nm laser.

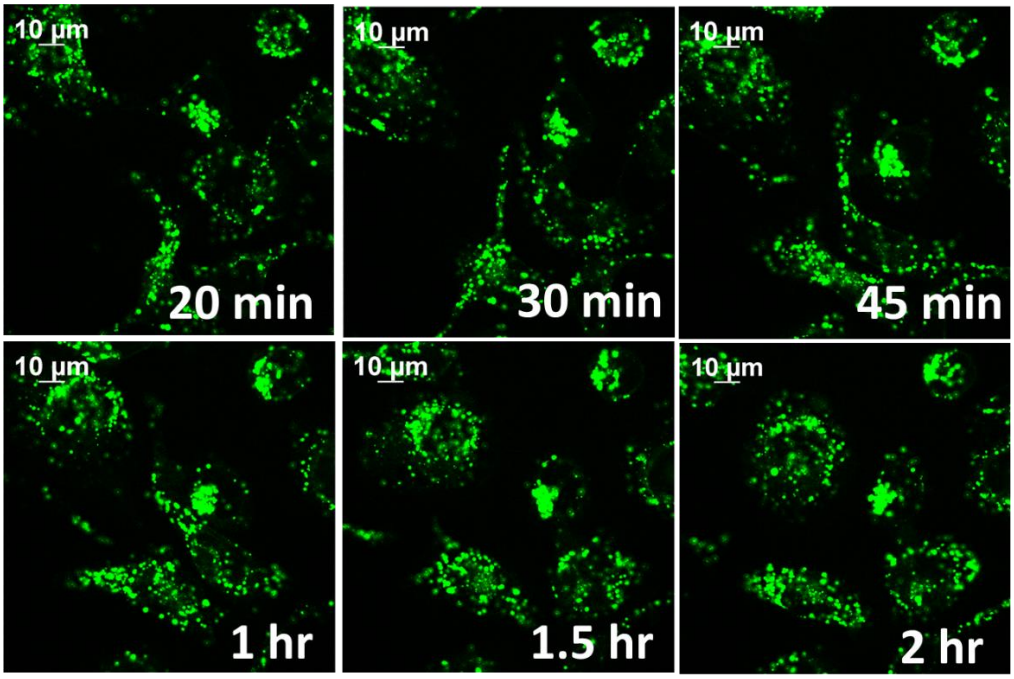


Figure S9. Confocal microscope time study images of MO3.13 cells stained with 0.2 µM LysoTracker Green® at 100x magnification. Compound was excited with 488 nm laser.

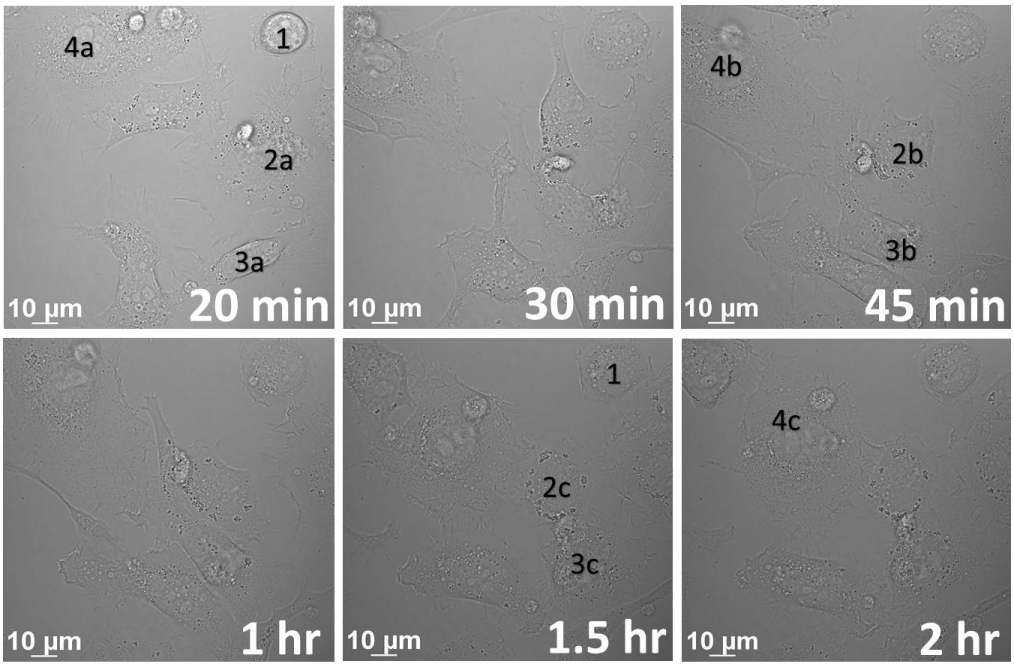
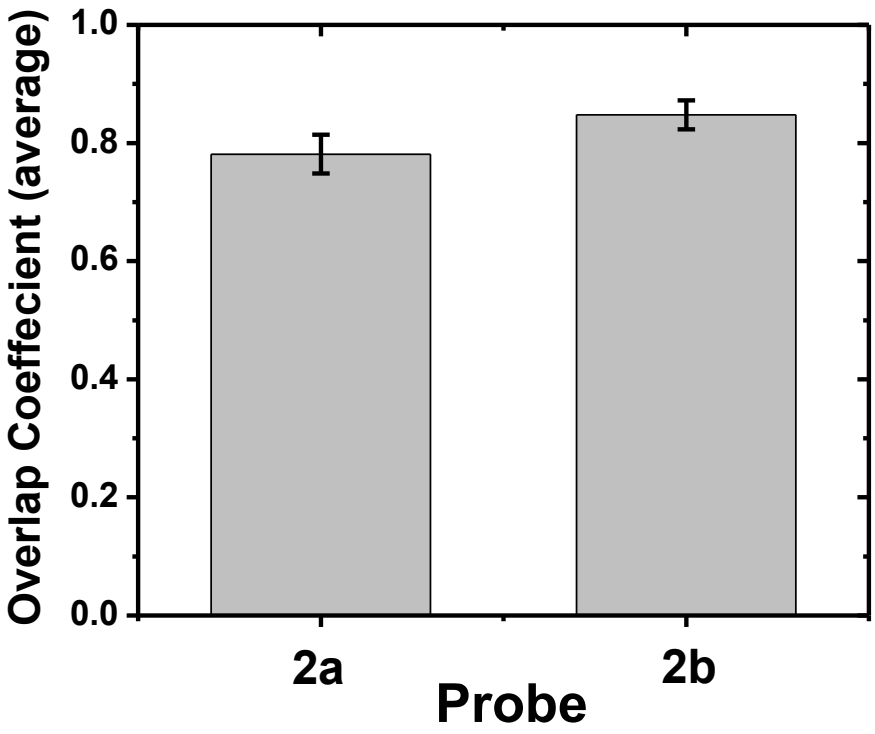


Figure S10. Bright field from Confocal microscope time study images of MO3.13 cells stained with 0.2 µM LysoTracker Green® at 100x magnification. Compound was excited with 488 nm laser. Morphology changes with time as follows: 1 – Round cell (dead cell); 2a – Healthy cell, 2b – Morphology changed significantly, 2c – Shrinking and round up cell; 3a – Healthy cell, 3b – Healthy cell, 3c – Shrinking and round cell; 4a – Healthy cell, 4b – Healthy cell, 4c – Shrinking cell.



Probe	Average Overlap coefficient	Error +/-
2a	0.7812	0.033
2b	0.8476	0.025

Figure S11. Mander's overlap coefficient calculations for **2a** and **2b** in colocalization experiments with LysoTracker Red DND 99 in NHLF and MO3.13 cells.

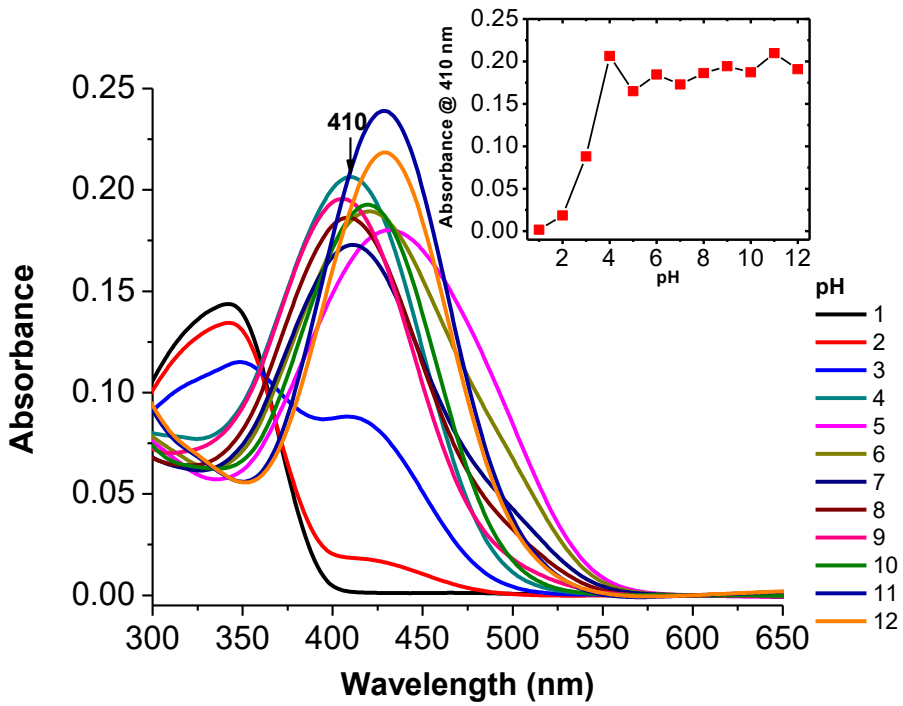


Figure S12. UV-vis absorption of **2a** at different pH at room temperature.

pH	Quantum yield
1	0.075
2	0.0085
3	0.0051
4	0.004
5	0.0041
6	0.0057
7	0.0059
8	0.019
9	0.15
10	0.21
11	0.49
12	0.61

Figure S13. Quantum yield values for **2a** at different pH in room temperature.

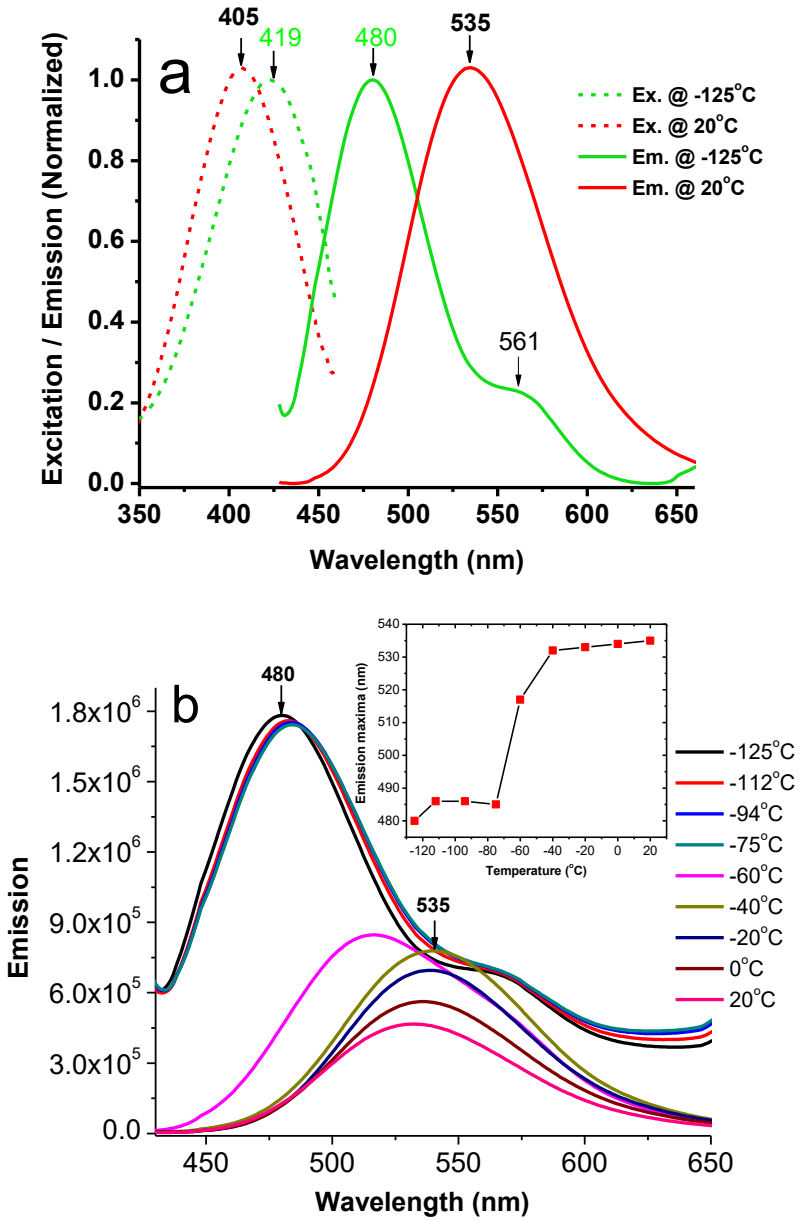


Figure S14. (a) Normalized emission of **2a** in ethanol solvent at different temperature. (b) emission of **2a** at various temperature. The emission at 480 nm is likely from the flavonoid's normal form (N*), while the emission at ~535 nm from the tautomeric form (T*). The inset plots emission maxima vs. temperature °C.

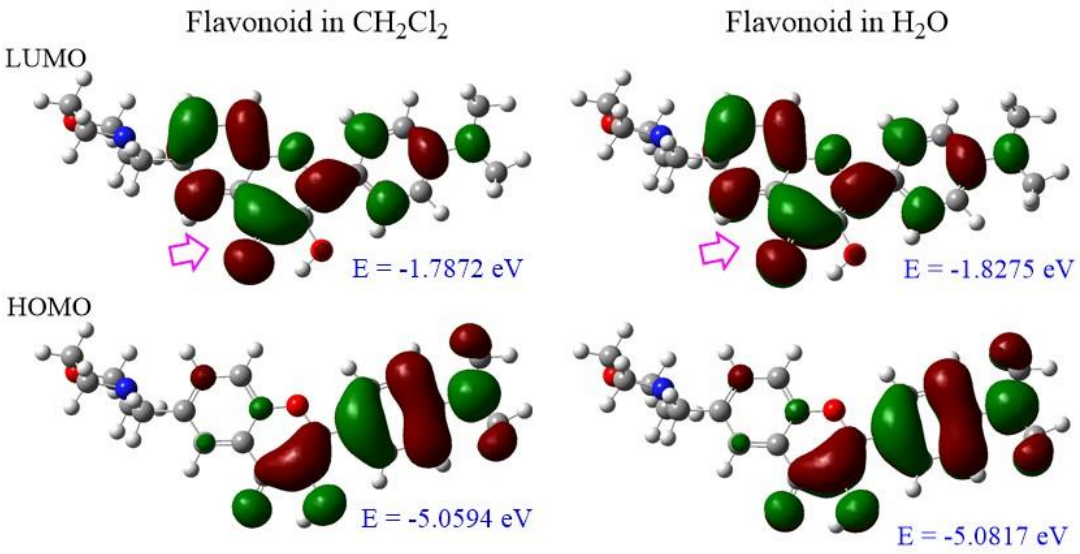


Figure S15. Molecular HOMO and LUMO orbitals of **2a** in CH_2Cl_2 and water solvents. The orbitals were generated by using Gaussian 09 software with DFT at B3LYP/6-31(d,p) setting, and with geometry optimization followed by energy minimization. In the LUMO orbitals, the C=O group (indicated by a double arrow) exhibits a slightly larger electron density in H_2O than in CH_2Cl_2, attributing to stronger solvent effect in water.

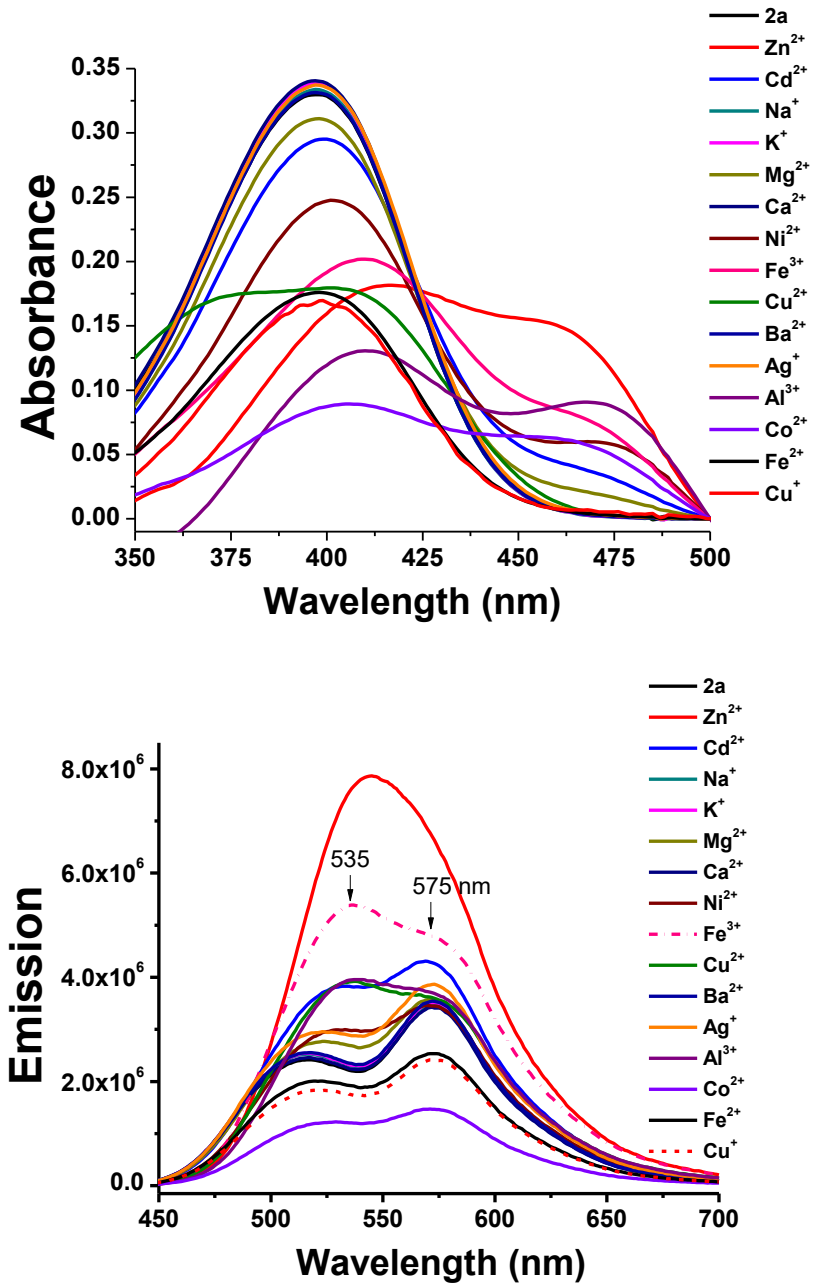


Figure S16. Absorption (a) and Fluorescence emission (b) responses of probe **2a** (10 µM in acetonitrile) upon addition of different metal ions (10 µM in water) at room temperature. The emission spectra were obtained by exciting metal species added probe at 400nm.

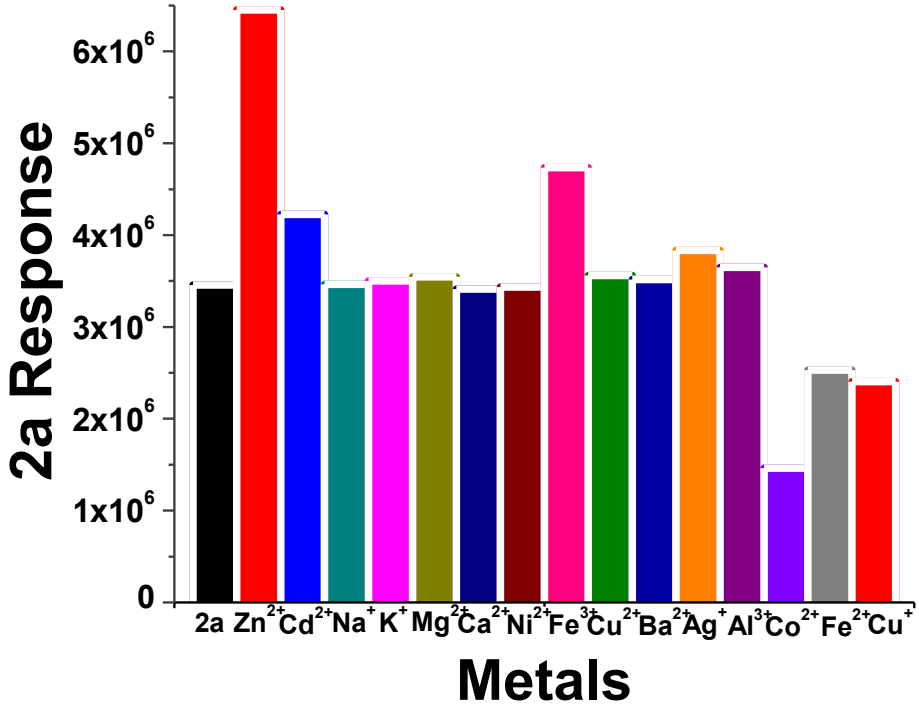


Figure S17. Emission intensity of probe **2a** (10 µM in acetonitrile) at 575 nm upon addition of different metal ions (10 µM in water) at room temperature. The emission spectra were obtained by exciting metal species added probe at 400nm.

Note (1): The slight fluorescence enhancement should not be a concern for cell application, as intracellular Zn^{2+} concentration is typically in a much lower concentration (e.g. nanomolar range).

Note (2): It should be noted that the probe **2a** has two emission peaks at ~535 nm (from N*) and 575 nm (from T*) in aqueous (see Figure S16). The tautomer emission (T*) wavelength at 575 nm was used to generate the plot here, since the emission wavelength at 575 nm appeared to be more consistent in comparison with the wavelength at ~535 nm.

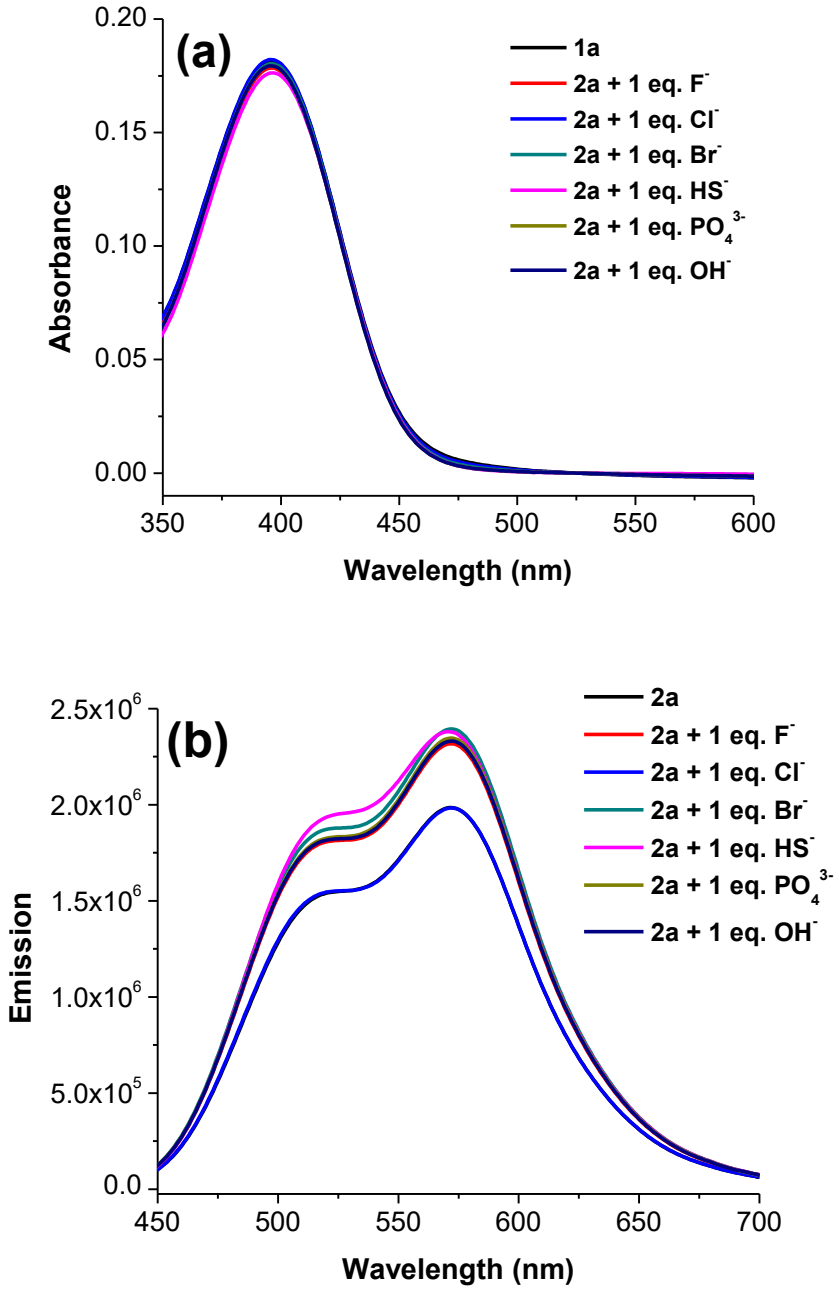


Figure S18. Absorption (a) and Fluorescence emission (b) responses of probe **2a** (10 µM in acetonitrile) upon addition of different 1 eq. of anions ions (10 µM in water) at room temperature. The emission spectra were obtained by exciting metal species added probe at 400nm. Tetrabutylammonium (R_4N^+) salts were used to prepare anion solutions.

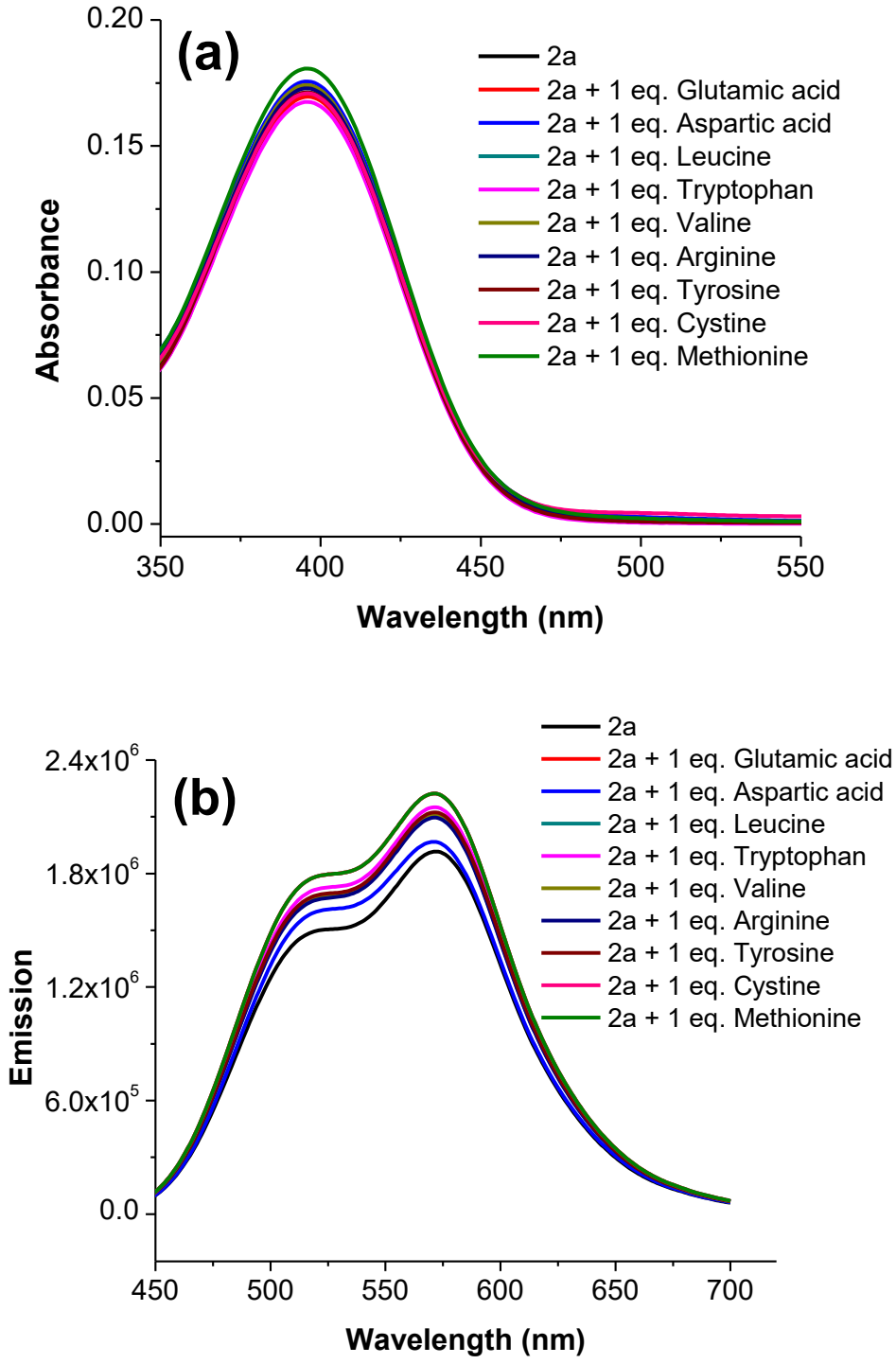


Figure S19. Absorption (a) and Fluorescence emission (b) responses of probe **2a** (10 µM in acetonitrile) upon addition of different 1 eq. different amino acids (10 µM in water) at room temperature. The emission spectra were obtained by exciting metal species added probe at 400nm.

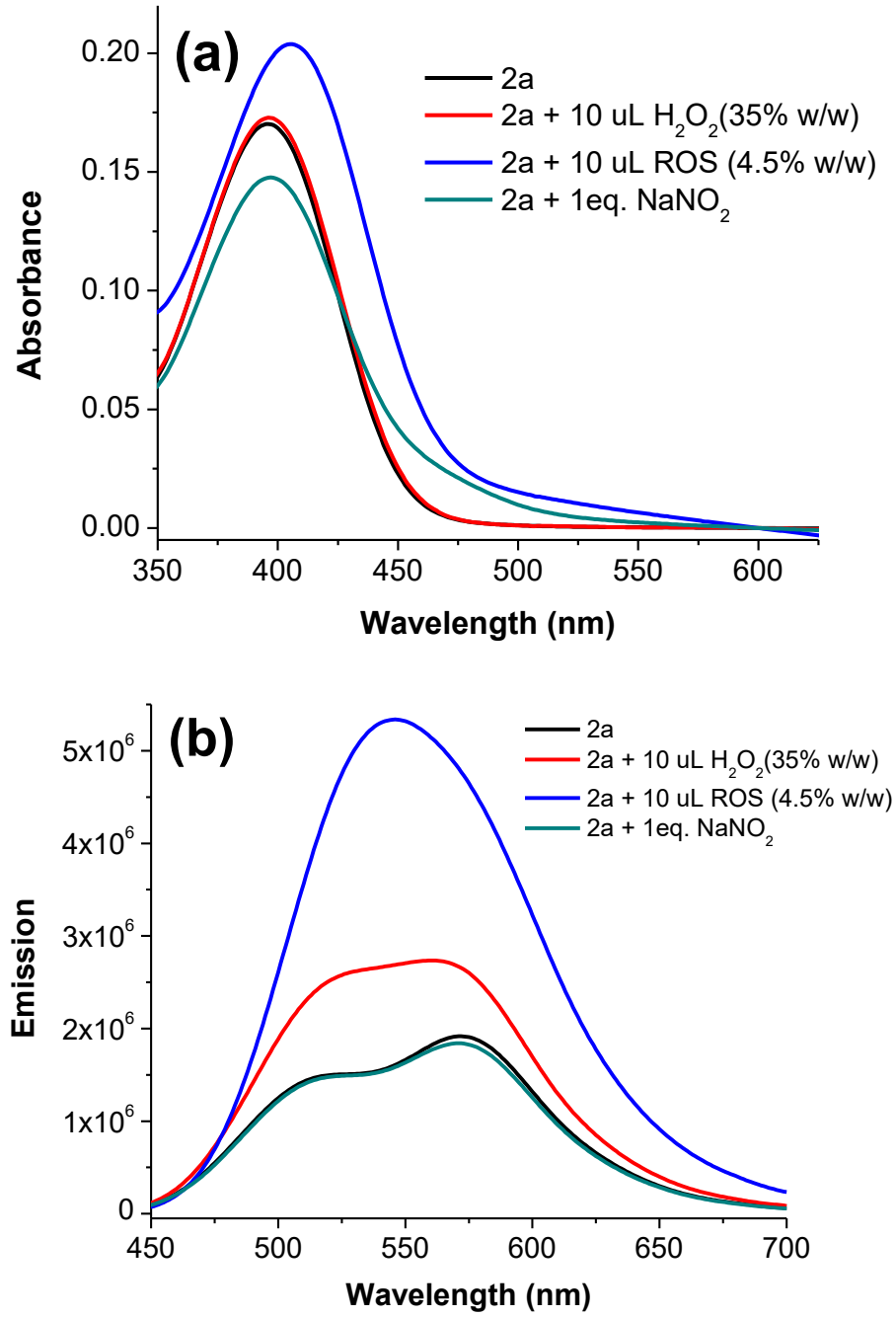


Figure S20. Absorption (a) and Fluorescence emission (b) responses of probe **2a** (10 µM in acetonitrile) upon addition of different reactive oxygen and nitrogen species at room temperature. The emission spectra were obtained by exciting probe at 400 nm, after addition of reactive oxygen species (ROS) or reactive nitrogen species. Two different ROS were used. Potassium peroxomonosulphate compound (~ 4.5 % active oxygen) and hydrogen peroxide (30% w/w) were prepared at 10 mM concentration. the reactive nitrogen species (HNO_2) was prepared from $H^+/NaNO_2$ solutions at 10 mM concentration ($2HNO_2 \rightarrow NO_2 + \mathbf{NO} + H_2O$).

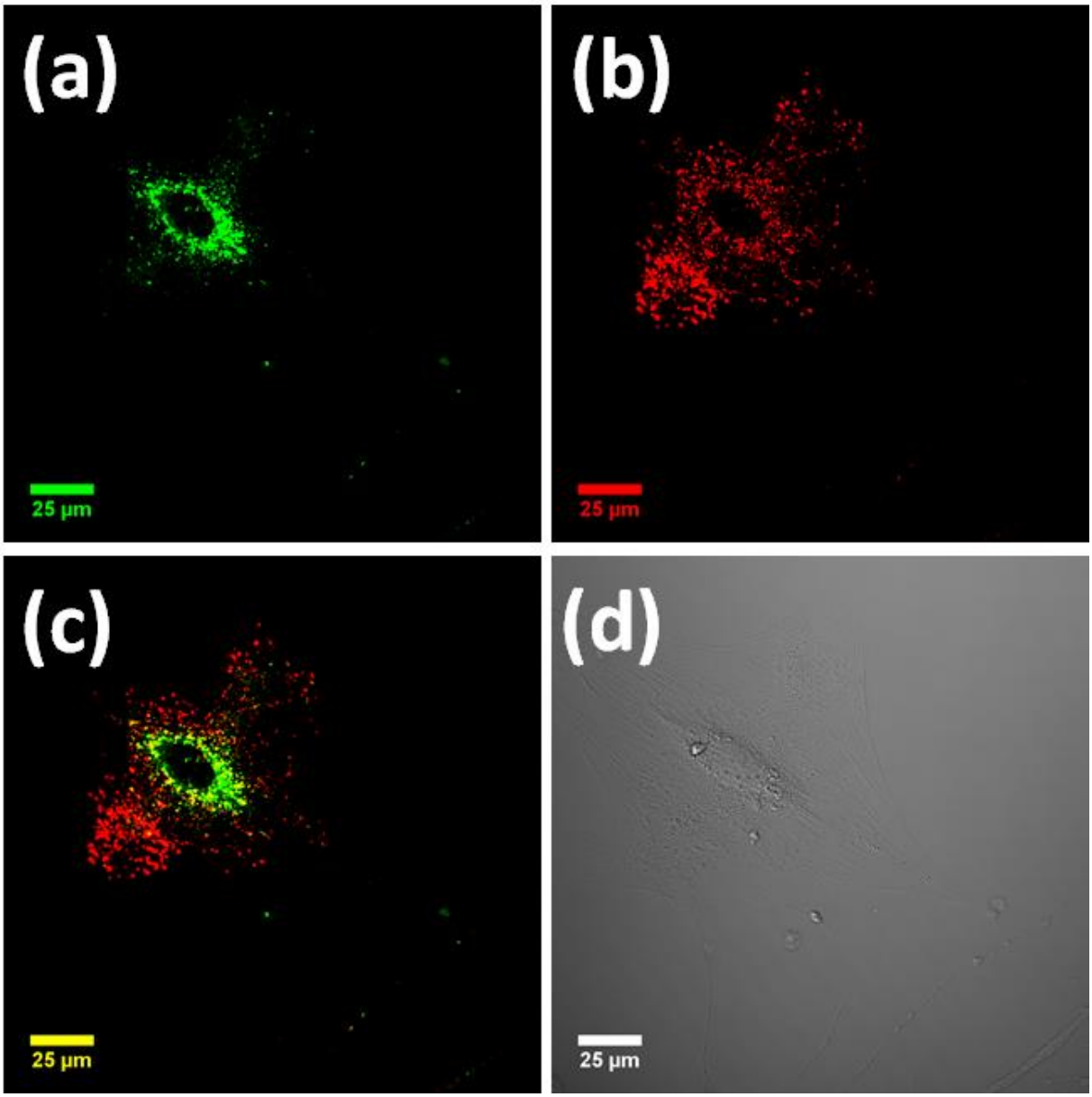


Figure S21. Confocal microscope images of NHLF cells stained with 2 µM **2a** for 30 minutes (a) and sequential sating with pHrodo^{TM} Red Avidin (5 µg/ml) for 1 hour at 0° C and 30 minutes at 37° C (b). Figure (c) represents the overlapped image obtained for two dyes and figure (d) represents the bright field. Probe **2a** was excited with 405 nm laser and pHrodo^{TM} Red Avidin was excited with 561 nm laser.

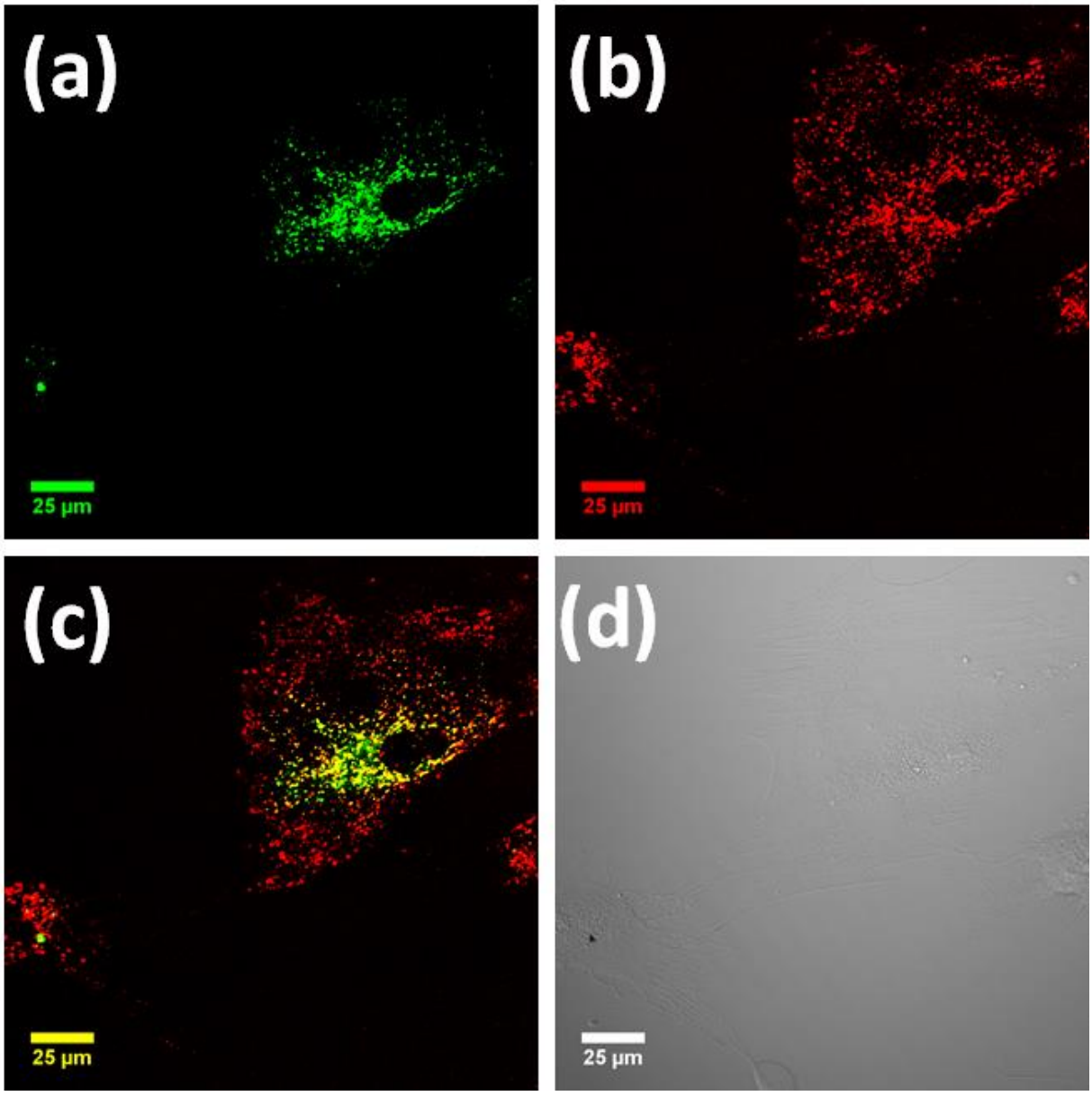


Figure S22. Confocal microscope images of NHLF cells stained with 2 µM **2b** for 30 minutes (a) and sequential sating with pHrodo^{TM} Red Avidin (5 µg/ml) for 1 hour at 0° C and 30 minutes at 37° C (b). Figure (c) represents the overlapped image obtained for two dyes and figure (d) represents the bright field. Probe **2b** was excited with 405 nm laser and pHrodo^{TM} Red Avidin was excited with 561 nm laser.

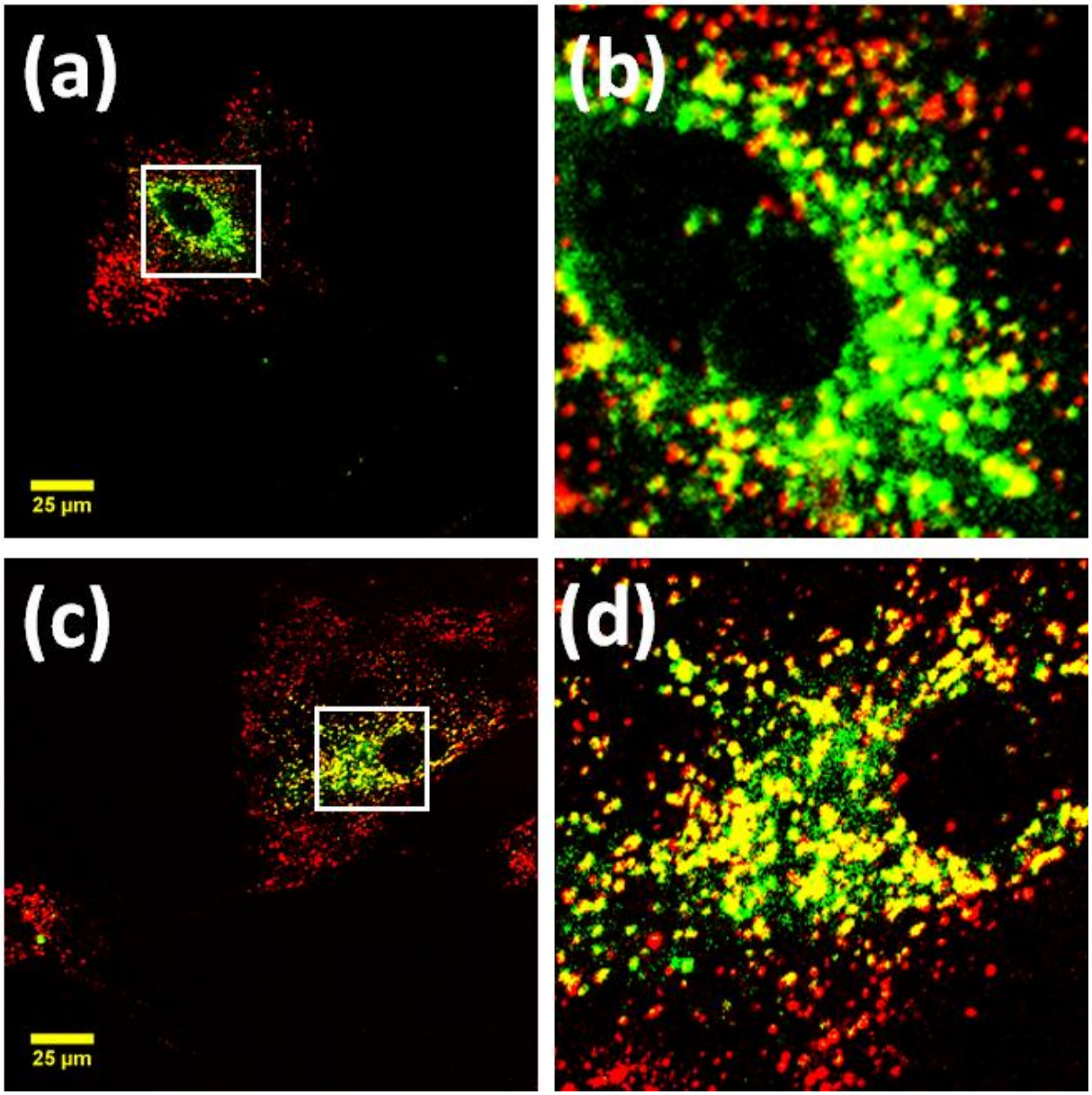


Figure S23. Figures (a) and (c) represents overlapped fluorescent confocal images obtained for pHrodo^{TM} Red Avidin in the presence of probes **2a** and **2b** at 2 µM concentration. Figure (b) and (d) represents the digitally zoomed in regions from images (a) and (c) to highlight the overlapped regions, respectively.

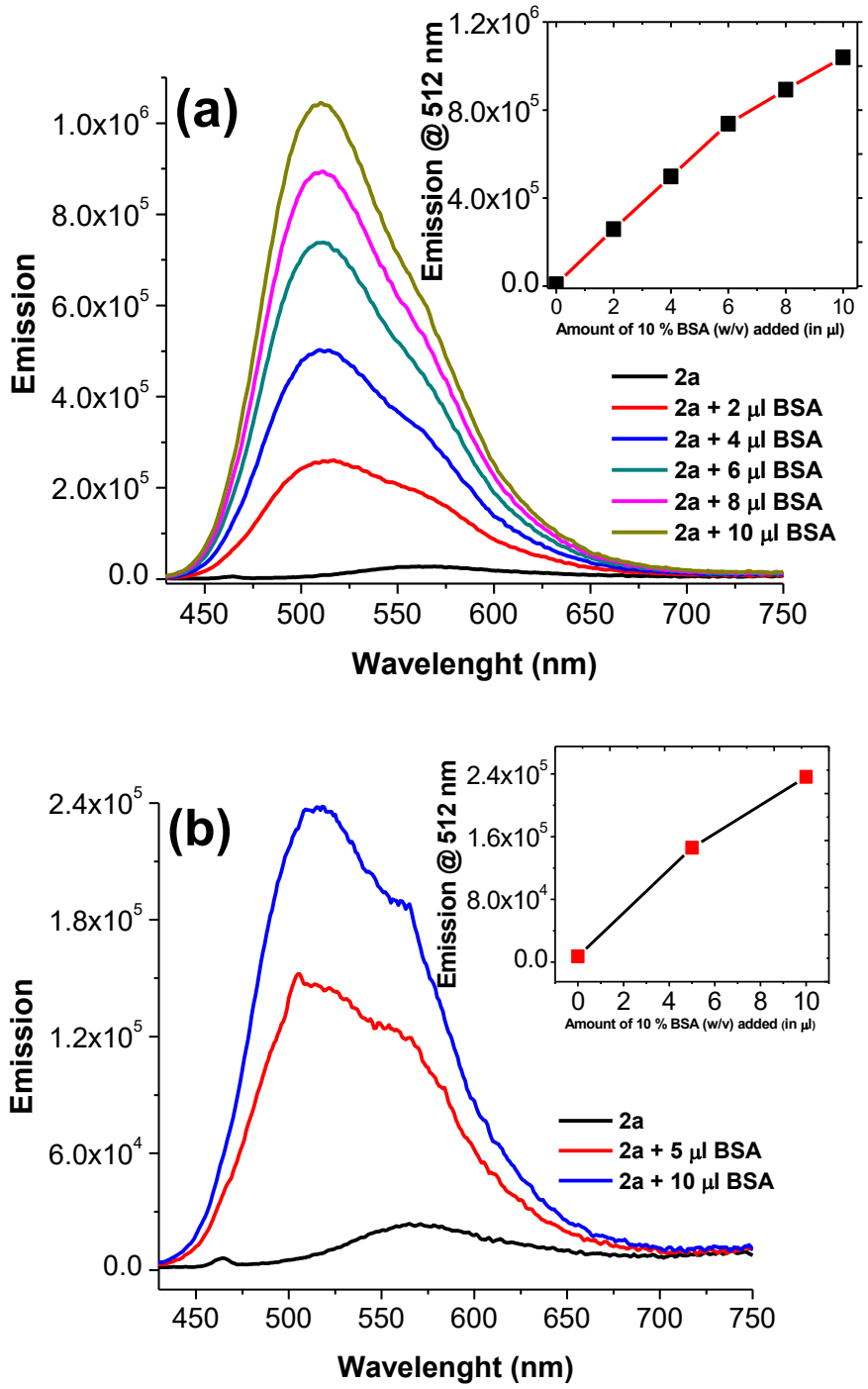


Figure S24.1 Fluorescence emission spectra obtained for **2a** (5 x 10^{-6} M) upon titration with 10% BSA (w/v) at room temperature. Figure (a) represents the emission spectra obtained in de-ionized water and figure (b) represents spectra obtained at pH 4.3.

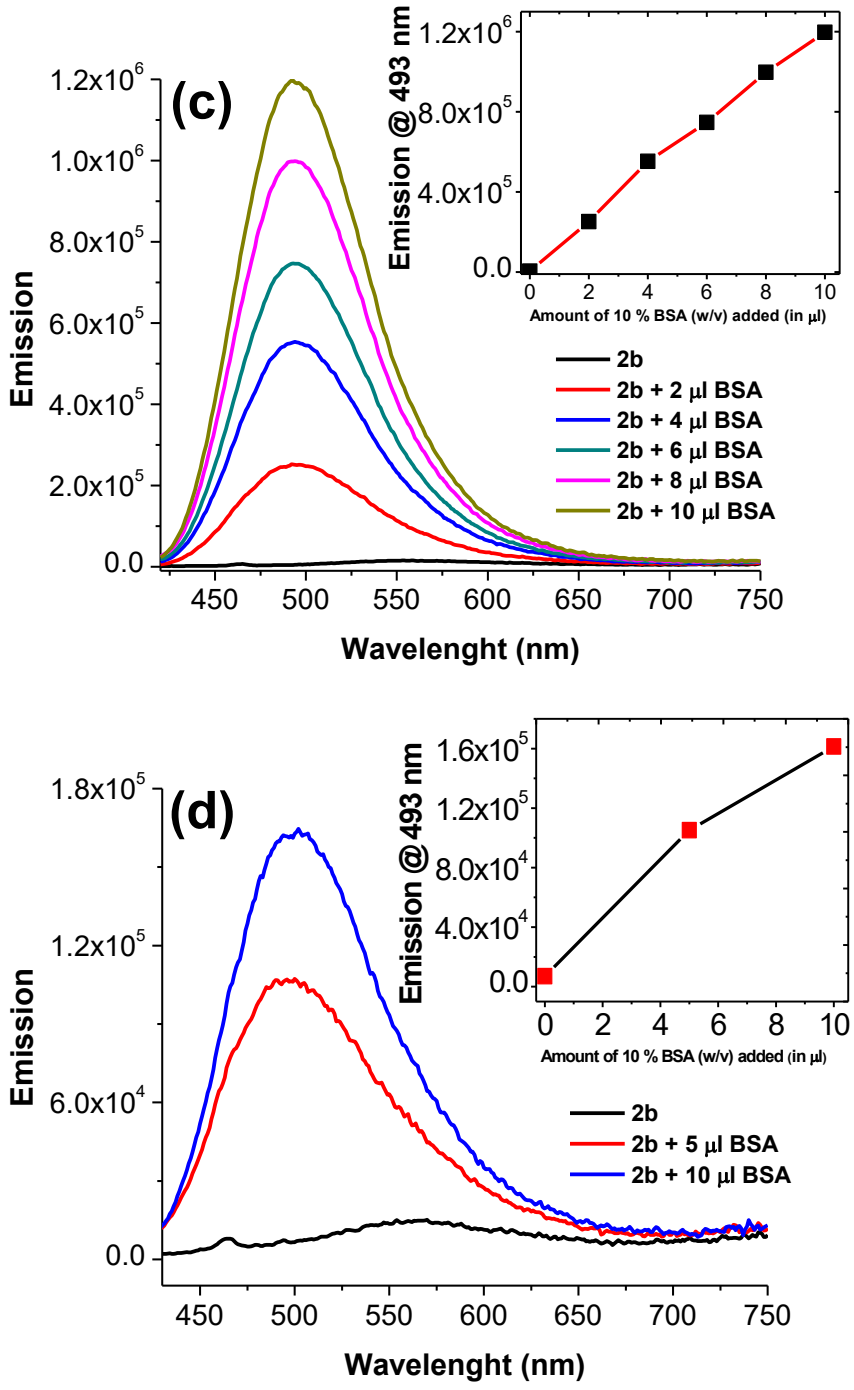


Figure 24.2 Fluorescence emission spectra obtained for **2b** (5 x 10^{-6} M) upon titration with 10% BSA (w/v) at room temperature. Figure (c) represents the emission spectra obtained in de-ionized water and figure (d) represents spectra obtained at pH 4.3.

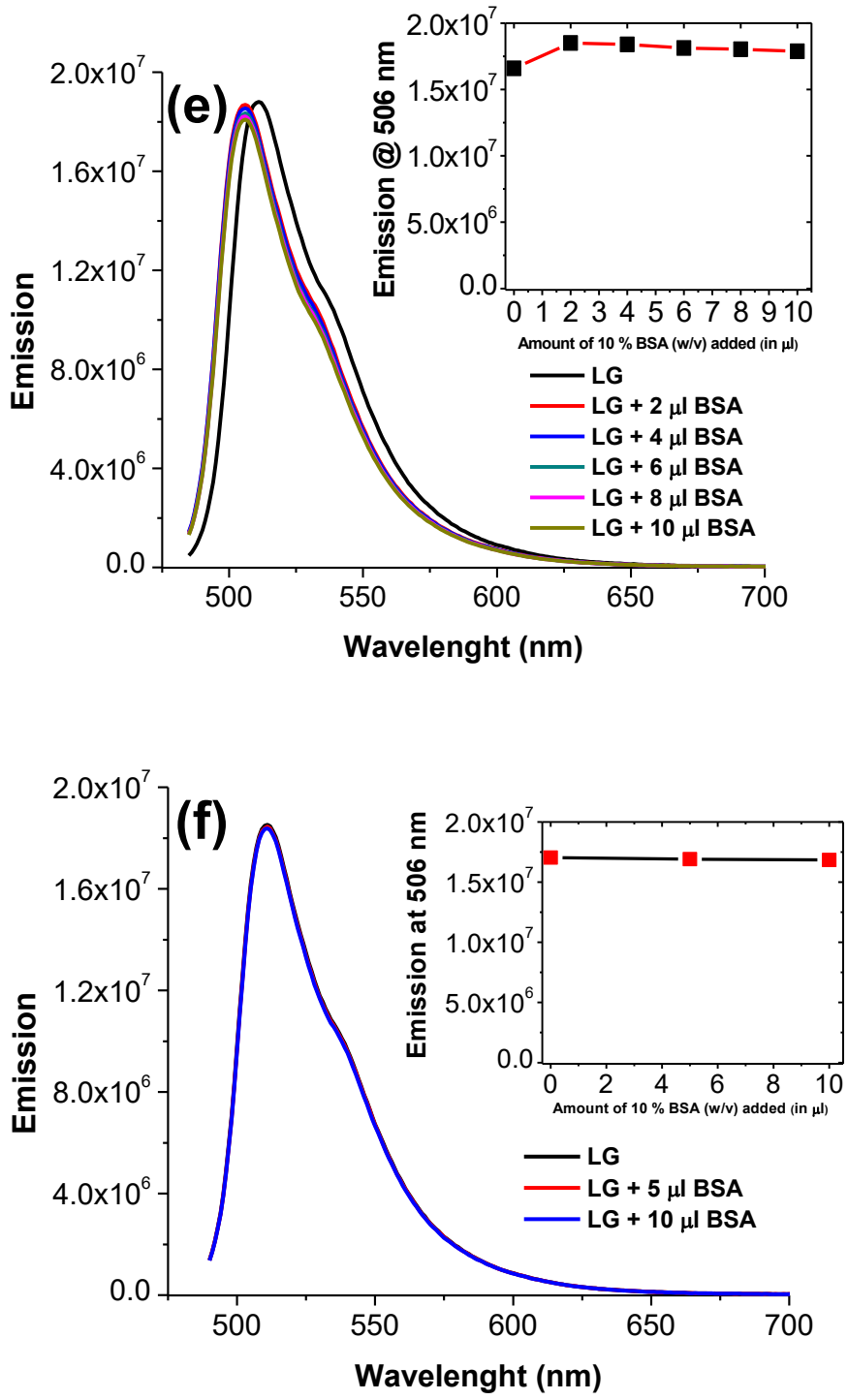


Figure 24.3 Fluorescence emission spectra obtained for **Lysotracker Green DND-26** (5 x 10^{-6} M) upon titration with 10% BSA (w/v) at room temperature. Figure (e) represents the emission spectra obtained in de-ionized water and figure (f) represents spectra obtained at pH 4.3.