Fluorescent Flavonoids for Endoplasmic Reticulum Cell Imaging

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1. Synthesis

2-(4-(diphenylamino)phenyl)-3-hydroxy-4*H*-chromen-4-one (7): 2'-hydroxyacetophenone (0.883 mL, 7.3 mmol) and 4-(diphenylamino)benzaldehyde (2.008 g, 7.3 mmol) were dissolved into the minimal amount of ethanol. A solution of potassium hydroxide (3.4 g in water) was added dropwise to the solution while on ice. The temperature was raised to 50°C for 12 hours yielding a dark red solution. Additional potassium hydroxide solution (3.1 g, 60.6 mmol, in water) was added, followed by hydrogen peroxide (30%, 6 mL) was added dropwise while on ice. The reaction was left to stir at room temperature for 24 hours yielding a dark orange mixture. The reaction was quenched with HCl (1 M), and ethanol removed via rotary evaporation. An orange precipitate was filtered and washed with water and a small amount of cold ethanol. The material was further purified by recrystallization from cold ethanol when needed. ^{1}H NMR (300 MHz, DMSO) δ 5.74 (s, 1H), 7.02-7.05 (d, 2H), 7.11-7.16 (t, 6H), 7.34-7.39 (t, 4H), 7.42-7.47 (t, 1H), 7.68-7.80 (m, 2H), 8.09-8.12 (m, 3H); ^{13}C NMR (75 MHz, DMSO) δ 21.27, 55.96, 109.54, 111.07, 114.72, 115.84, 117.77, 126.50, 136.87, 150.57

2-(4-(dimethylamino)phenyl)-3-hydroxy-6-methyl-4H-chromen-4-one (8): 2'-hydroxy-5'-methylyacetophenone (1.002 g) and 4-(dimethylamino)benzaldehyde (0.992 g) were dissolved into minimal amount of ethanol and a solution of potassium hydroxide (2.913 g in water) was added dropwise over 30 minutes while on ice. The temperature was raised to 50°C for 12 hours yielding a dark red solution. Additional potassium hydroxide solution (2.798 g in water) was added followed by hydrogen peroxide (30%, 5 mL) was added dropwise while on ice. The reaction was left to stir at room temperature for 24 hours yielding an orange solution. Reaction was quenched with HCl (1 M) and ethanol removed via rotary evaporation. Yellow/orange precipitate was filtered and washed with water and a small amount of cold ethanol. Material purified by

recrystallization from cold ethanol when needed. ^{1}NMR (300 MHz, DMSO) δ 2.43 (s, 3H), 3.00 (s, 6H), 6.82-6.85 (d, 2H), 7.57-7.63 (m, 2H), 7.86 (s, 1H), 8.08-8.12 (d, 2H), 9.06 (s, 1H);

2. Fluorescence Spectra and Imaging

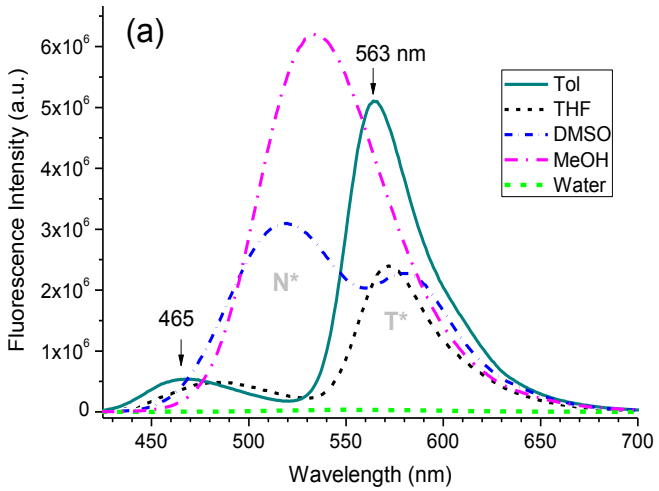


Figure S1. Fluorescence spectra of **2a** in different solvents at room temperature.

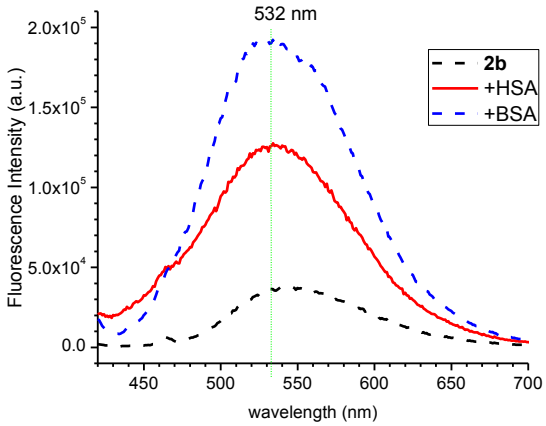


Figure S2. Fluorescence of **2b** (10 μM) in 1 mM HEPES buffer containing 1% DMSO, upon addition of HSA and BSA (10 μM) at room temperature.

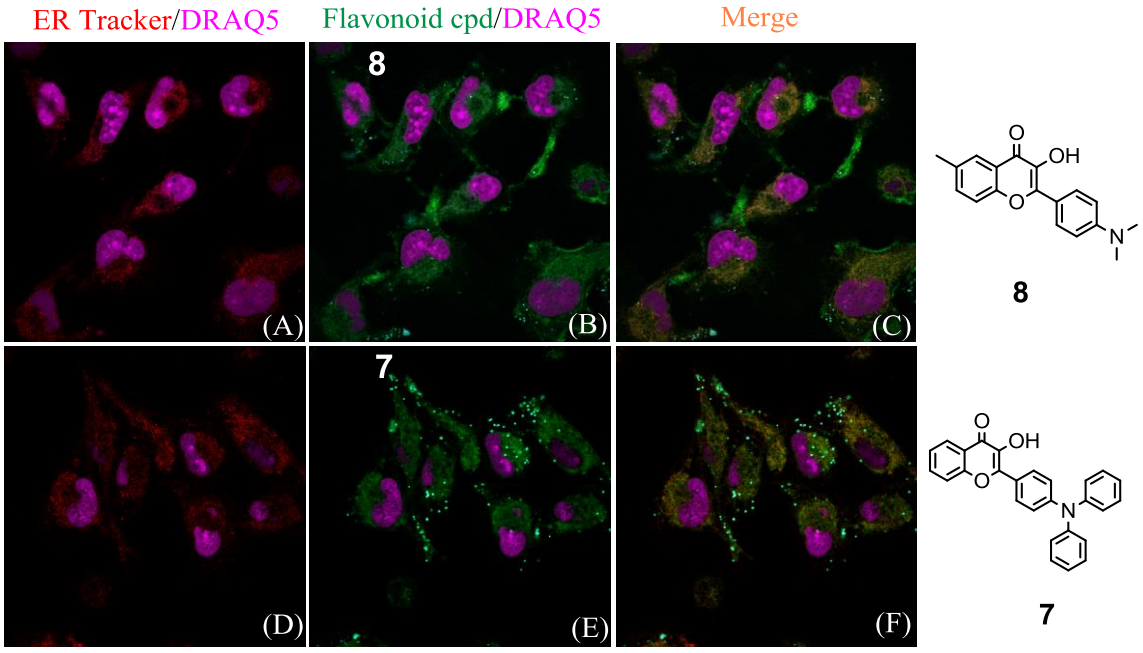


Figure S3. Confocal imaging of MO3.13 cells incubated with ER-Tracker (1 μM) (panels A and D), flavonoid compounds **8** (panel B) or **7** (panel E) (500 nM, for each dye respectively). Panels (C) and (F) are the merged images of ER Tracker Red and flavonoid probes. The nuclei of cells were stained with DRAQ5 (shown in purple). The green fluorescence from **7** and **8** did not match well with the ER-Tracker. The results pointed to that the amide group in flavonoid **2** plays an important role in ER-selectivity.

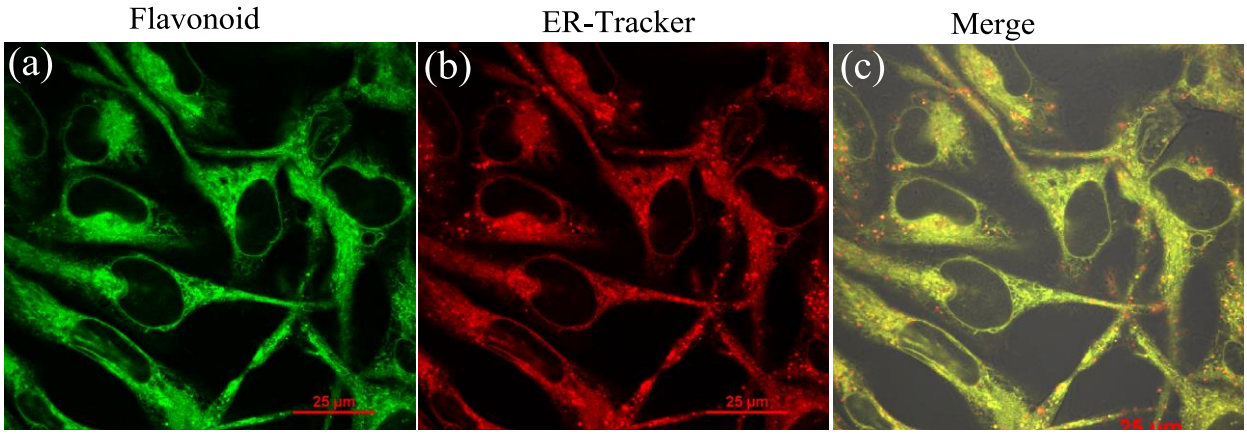


Figure S4. Confocal imaging of MO3.13 cells (oligodendrocyte cells) incubated with flavonoid **2b** (10 μM) (a) and ER-Tracker (1 μM) (b). The merged image (c) shows the good co-localization.

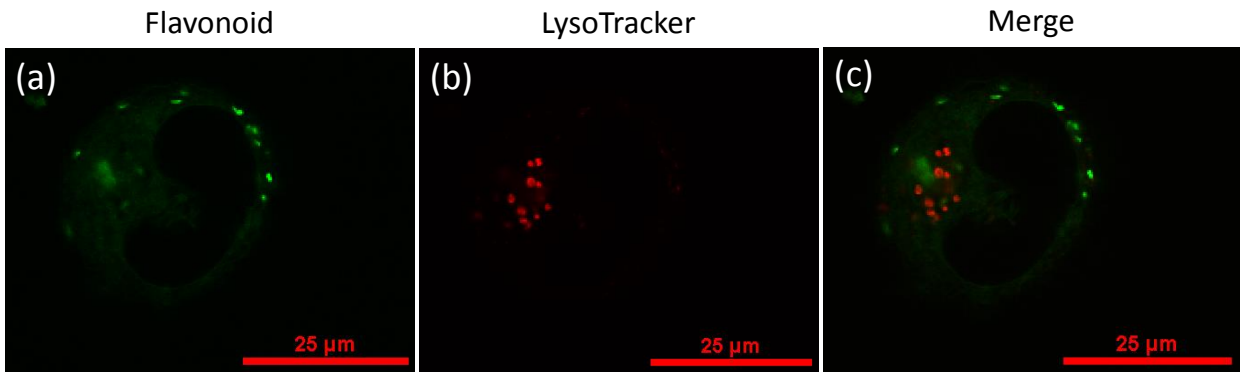


Figure S5. MO3.13 cells incubated with flavonoid **2b** (10 µM) (a) and LysoTracker (1 µM) (b). The merged image (c) shows no localization.

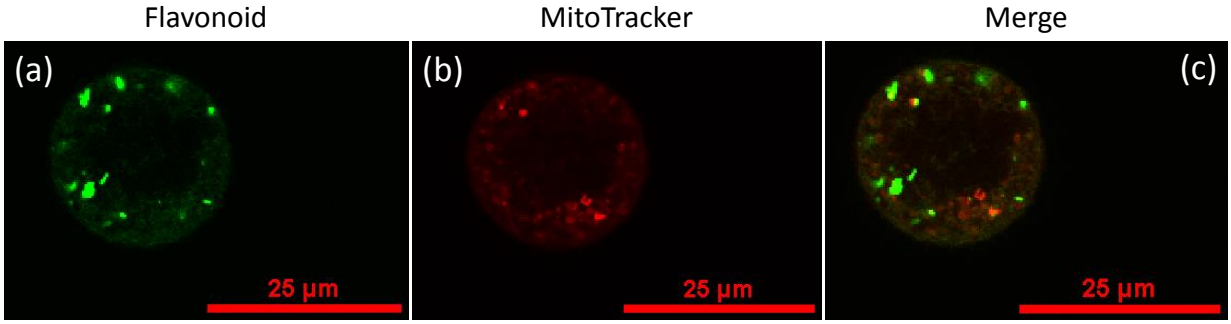


Figure S6. MO3.13 cells incubated with flavonoid **2b** (10 µM) (a) and MitoTracker (1 µM) (b). The merged image (c) shows no localization.

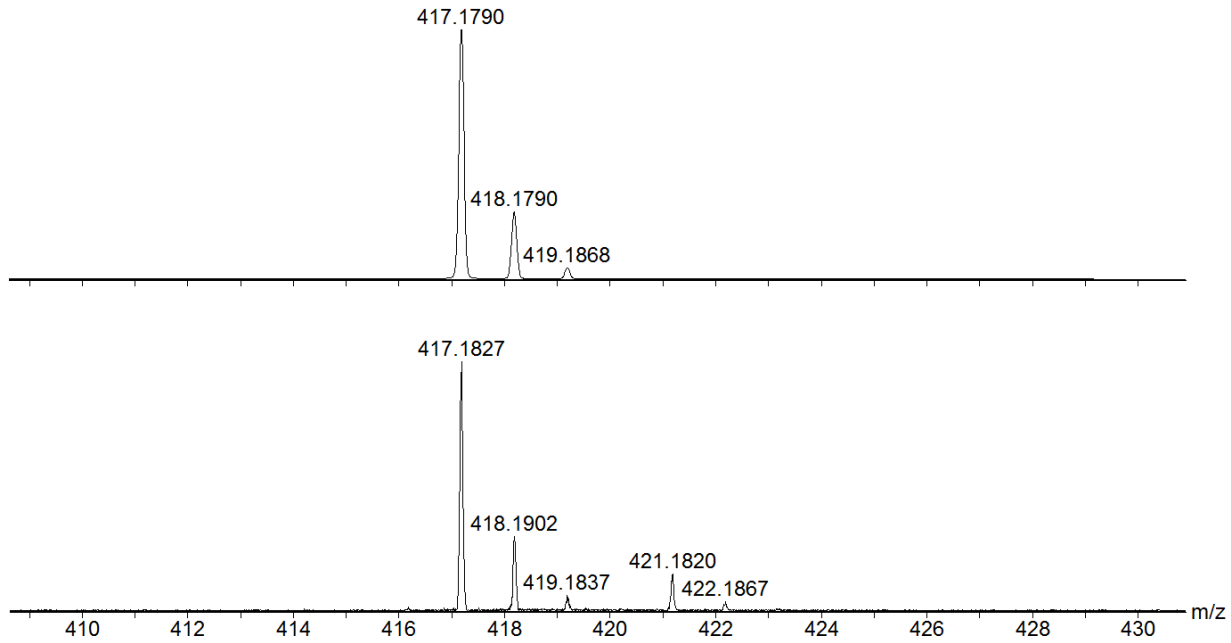


Figure S7. Theoretical (top) and actual spectrum (bottom) for **2a**.

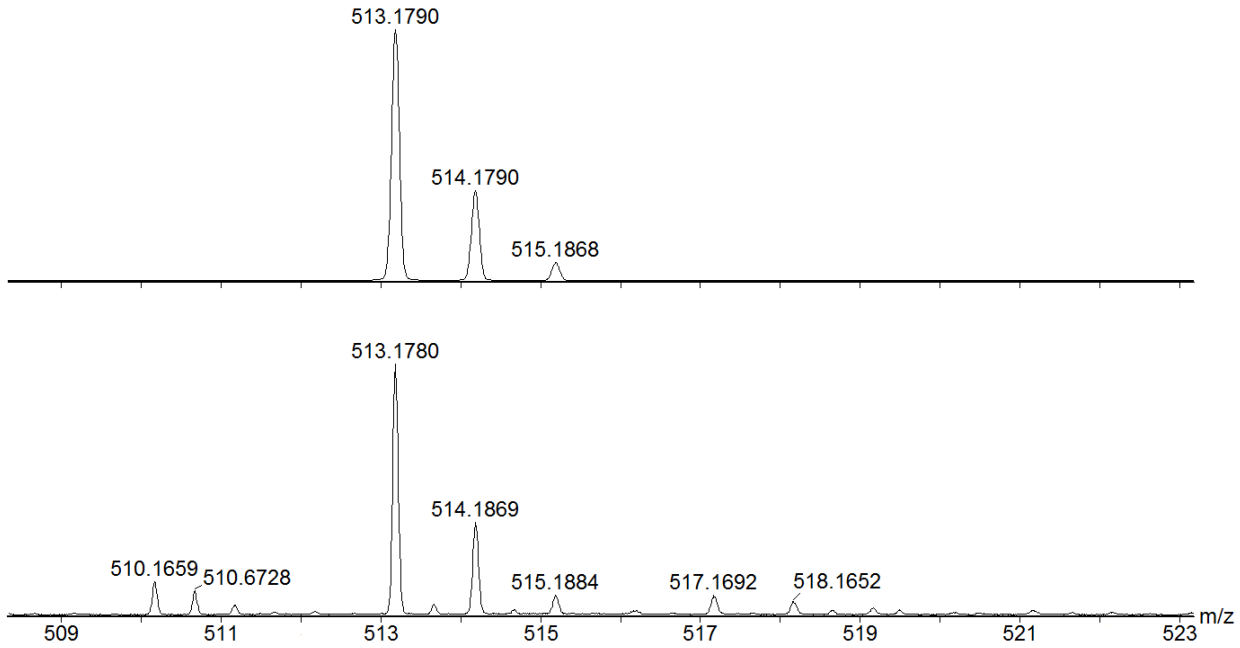


Figure S8. Theoretical (top) and actual (bottom) spectra of **2b**.

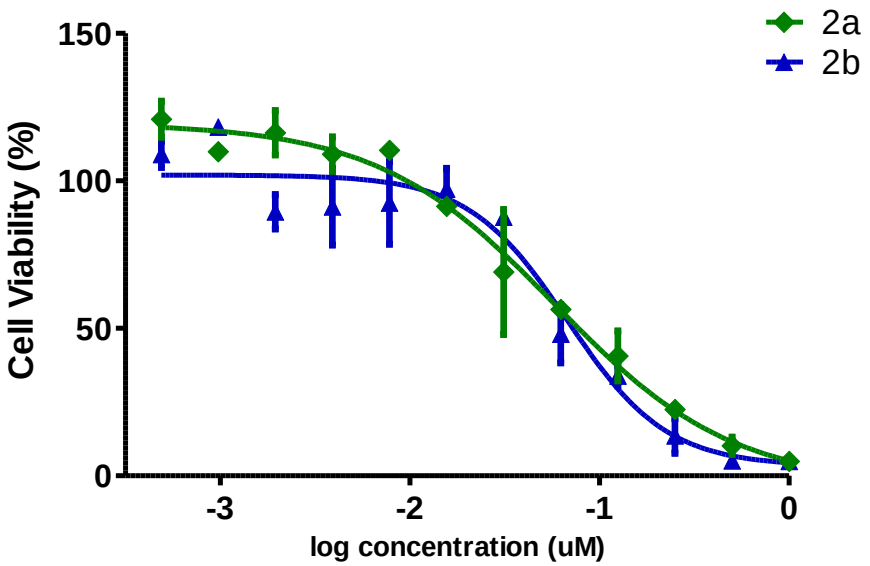


Figure S9: IC50 plots for **2a** and **2b** with MO3.13 cells treated for 24 hours, with mean IC50 values being 18.59 and 33.54 µM, respectively. The 95% confidence intervals are 11.99 to 28.80 µM for **2a** and 25.45 to 44.22 µM for **2b**. Cells were tested on two different occasions with all data combined for a total of n=6 for each concentration.

MTT calculations were performed using Graphpad Prism 5 software. All absorbance values were translated to percent viability based on the mean vehicle treated absorbance:

$$\frac{ABS_{Experiment}}{\mu(ABS_{Vehicle})} \times 100\%$$

Using the normalized cell percent viability data, the IC50 was then calculated by fitting the log of the concentration (µM) to the percent viability using a dose-response curve. Specifically, a nonlinear regression was used to fit the data to a log(inhibitor) vs. response (variable slope) curve:

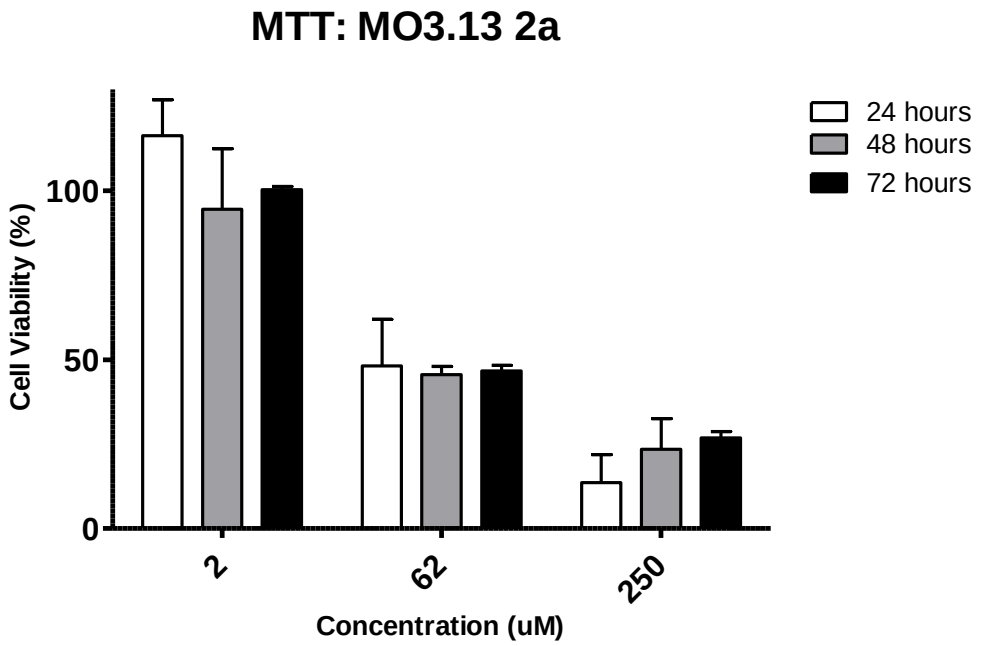
$$y = Bottom + \frac{(Top - Bottom)}{(1 + 10^{(logIC50 - x) * Hillslope)})}$$

IC50: is the concentration of agonist that gives a response half way between Bottom and Top.

HillSlope: describes the steepness of the family of curves.

Top and Bottom: are plateaus in the units of the Y axis.

a



b

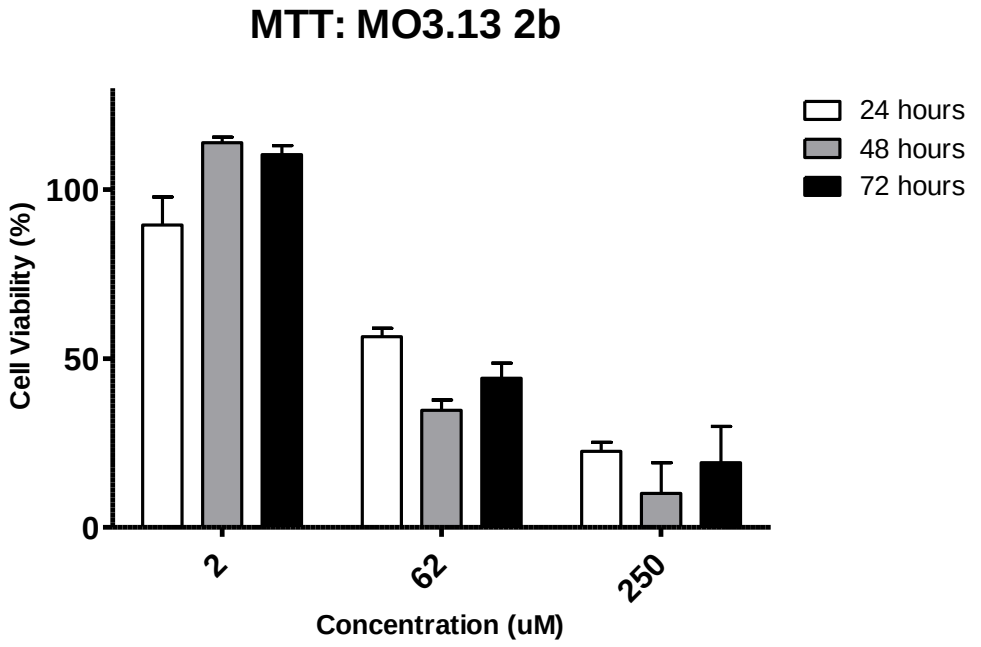


Figure S10: Kinetic cell viability plots for **2a (a)** and **2b (b)** with MO3.13 cells. Cells were treated with a range of concentrations for 24, 48, or 72 hours and the viability was measured via an MTT assay. The cells were tested in duplicate for each concentration at each time point.

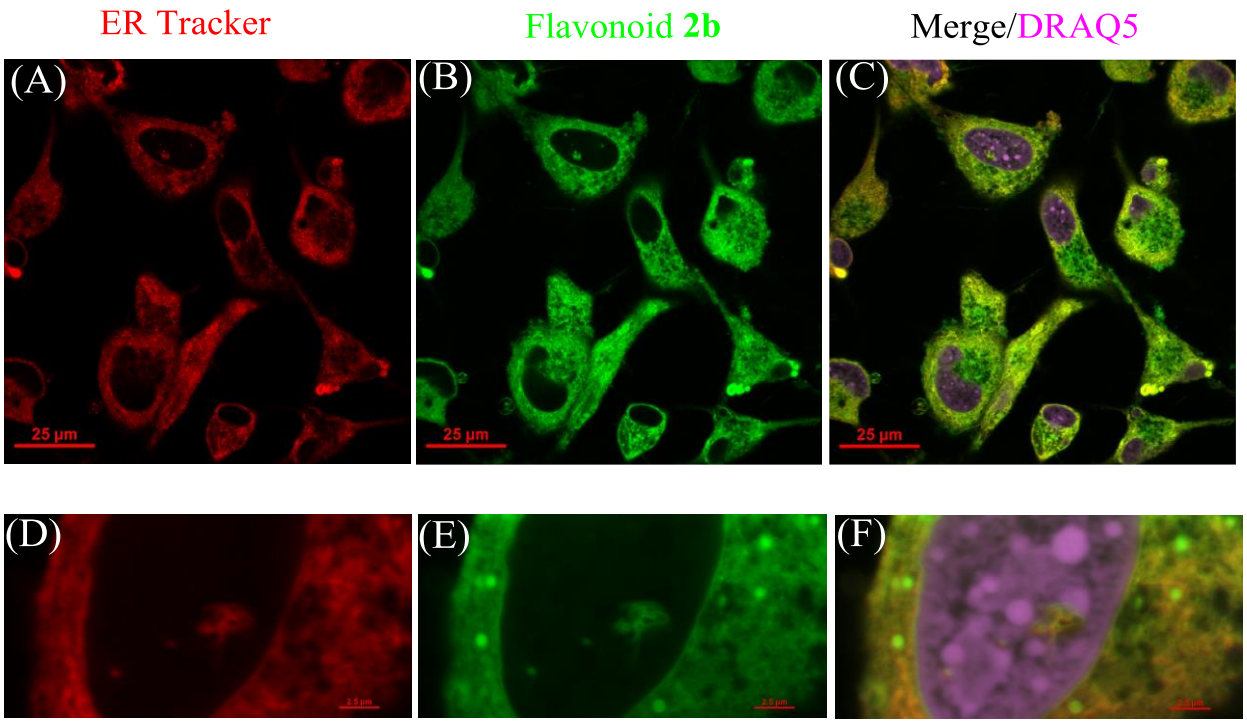


Figure S11: Confocal imaging of GL261 cells (astrocytes), which were incubated with ER-Tracker (1 µM, column A), flavonoid **2b** (500 nM, column B) for 30 minutes. Column C on the right is the merged images of ER Tracker Red, flavonoid dye, and nuclear dye DRAQ5 (purple). Magnification for top row is at 100x. For the bottom row, magnification is at 500x (digitally enhanced) and the scale bar is 2.5 µm.

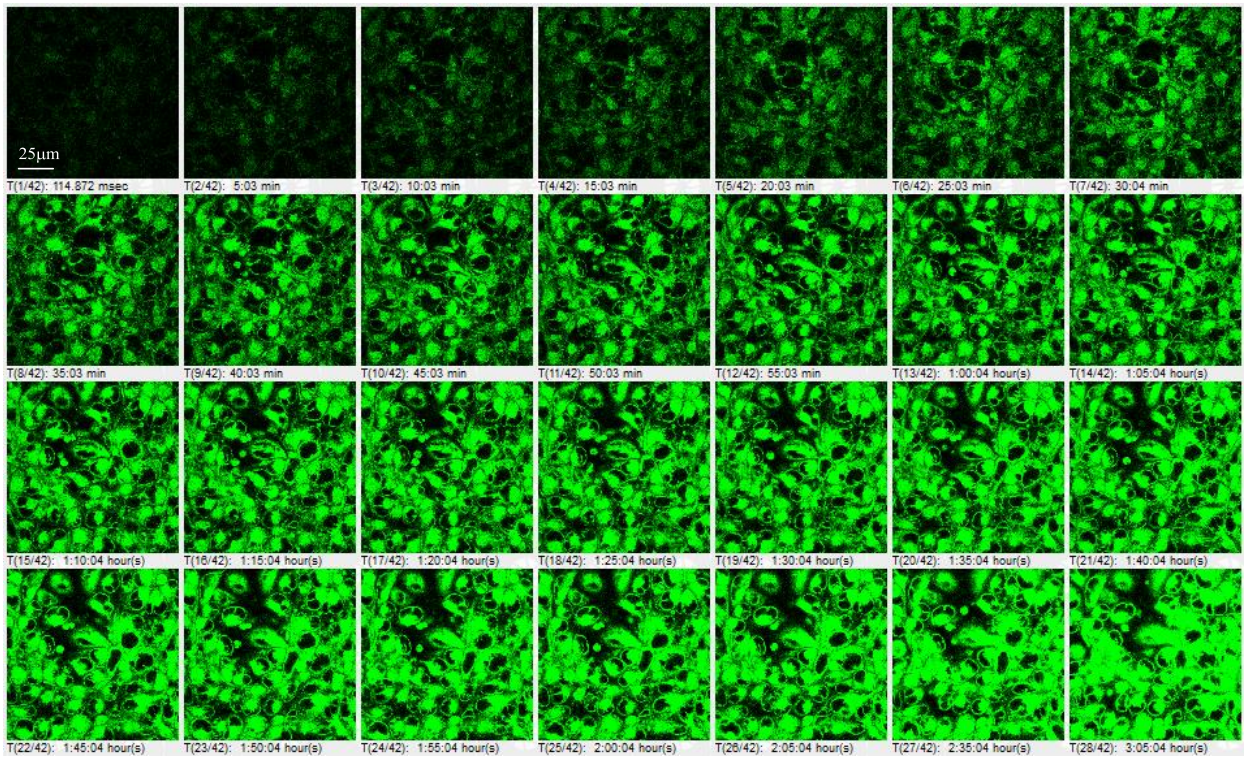


Figure S12. Confocal imaging of MO3.13 cells incubated with **2b** (1 µM). The images were acquired at 5 minute interval for 3 hours.

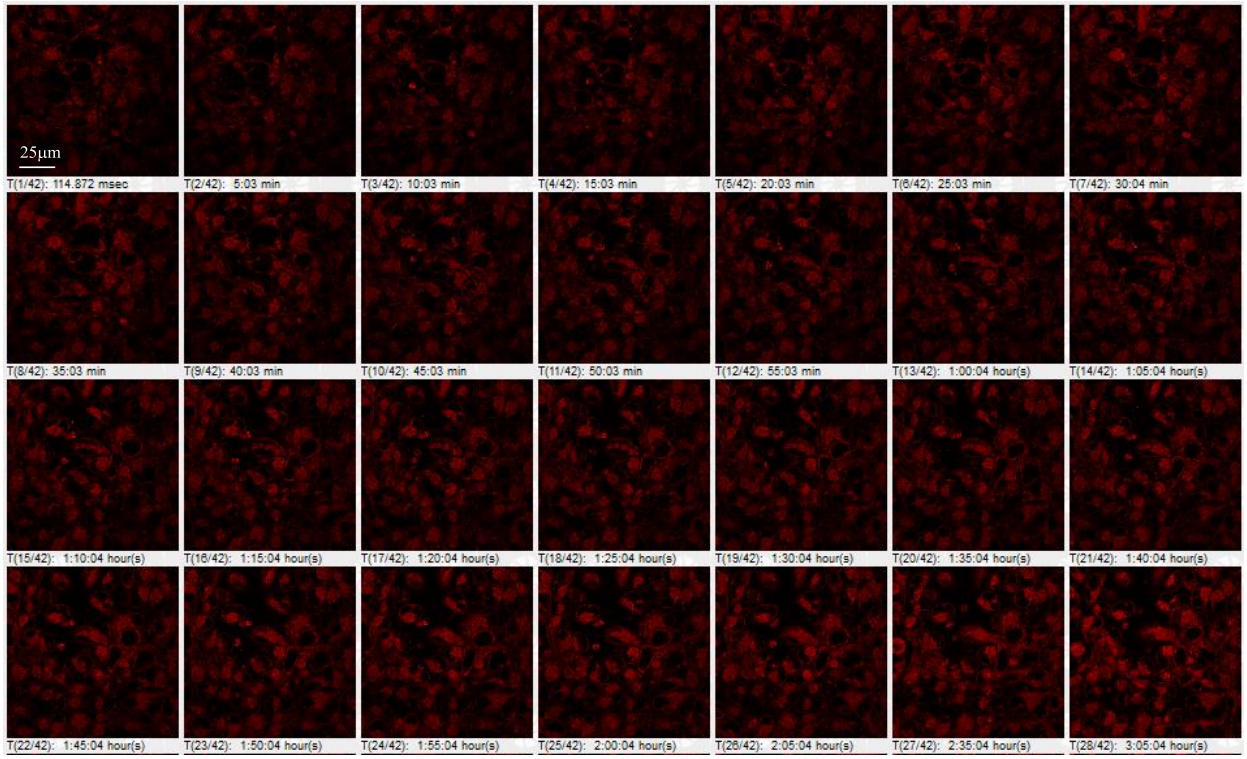


Figure S13. Confocal imaging of MO3.13 cells incubated ER-Tracker (1 µM). The images were acquired at 5 minute interval for 3 hours.