

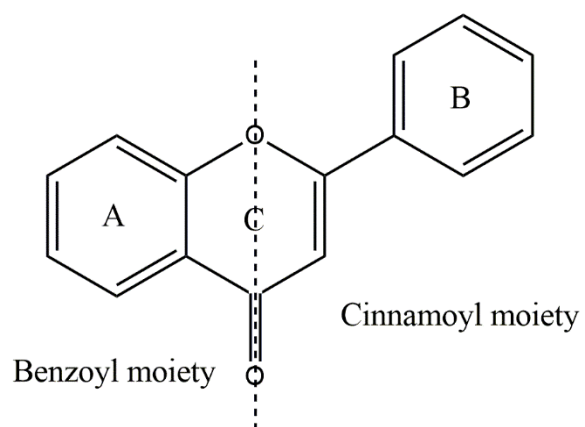
## **Supplementary Information**

### **Influence of Molecular Structures on Fluorescence of Flavonoids and Their Detection in Mammalian Cells**

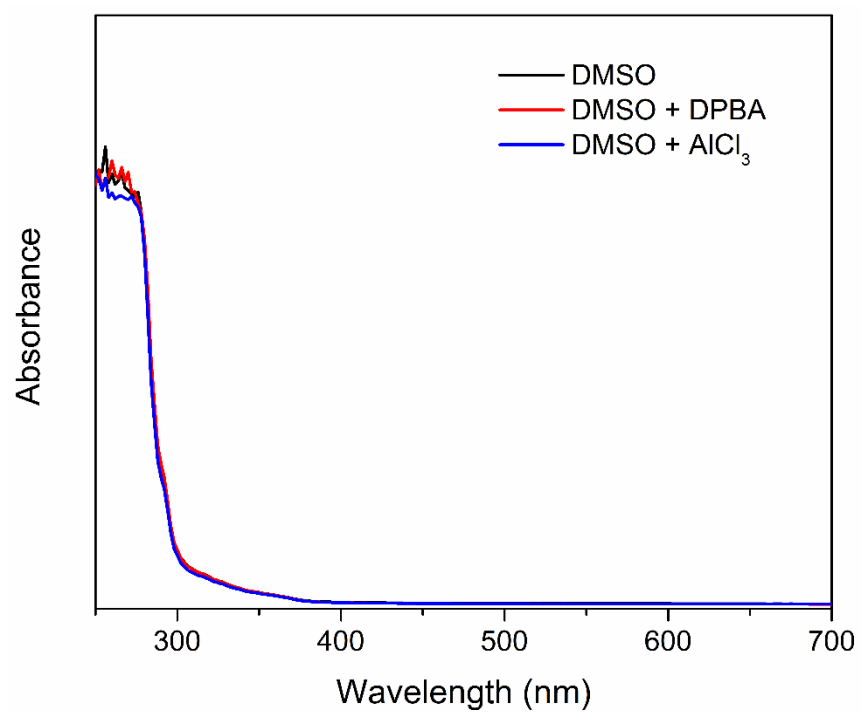
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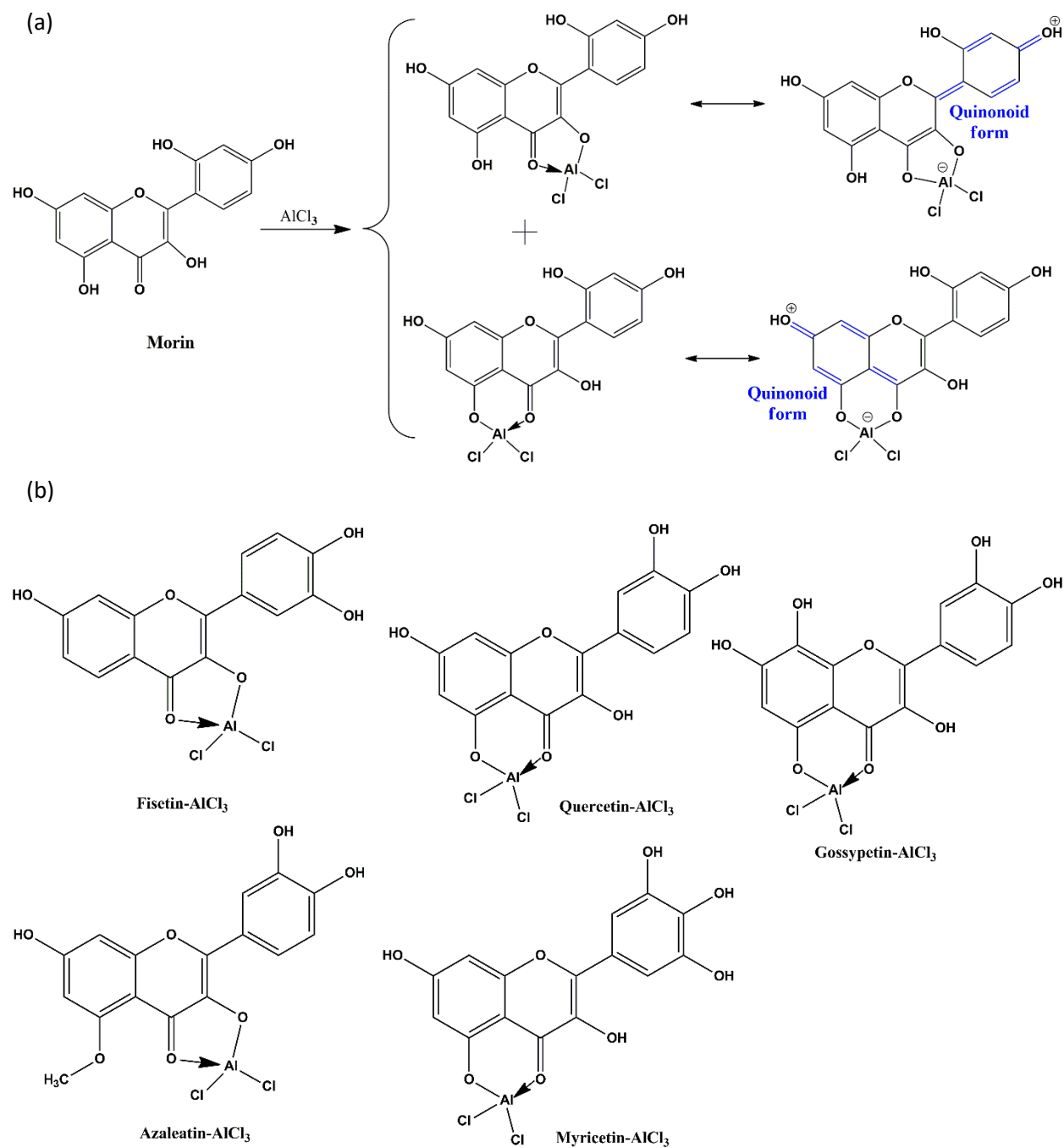
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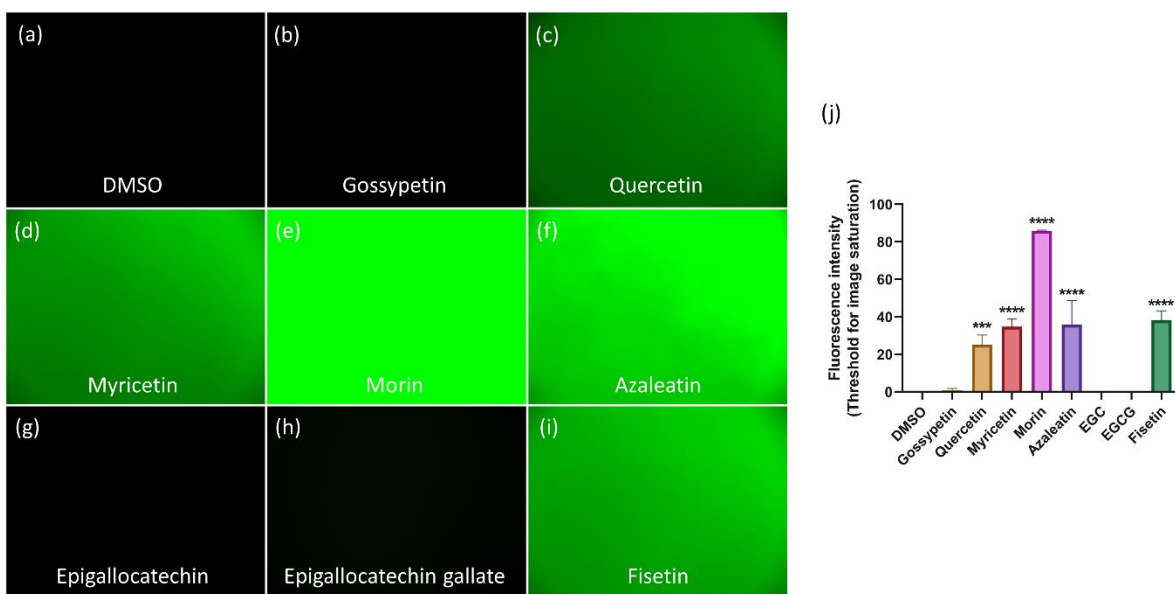
**Figure S1.** A schematic presentation of the basic flavonol structure comprised of the benzoyl and cinnamoyl moieties.



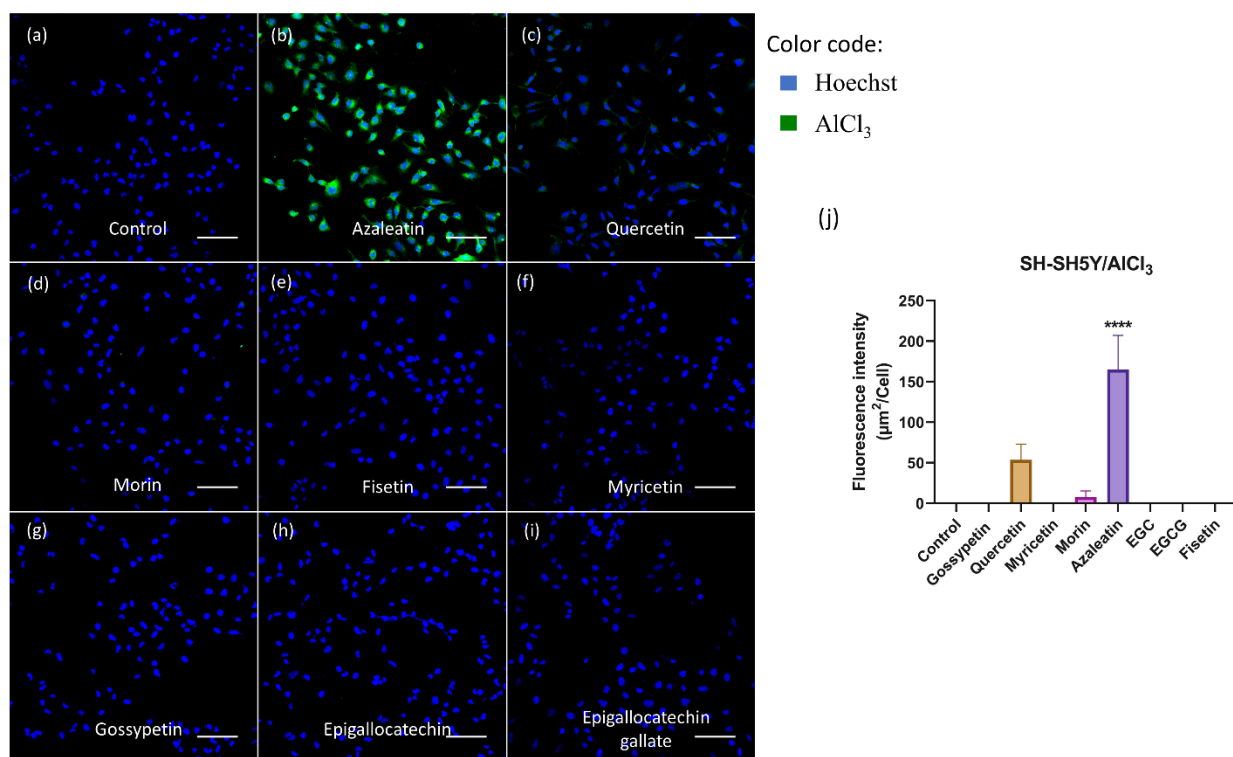
**Figure S2.** UV-visible spectra of DPBA and AlCl<sub>3</sub> in DMSO showing no peaks in the absence of flavonoids.



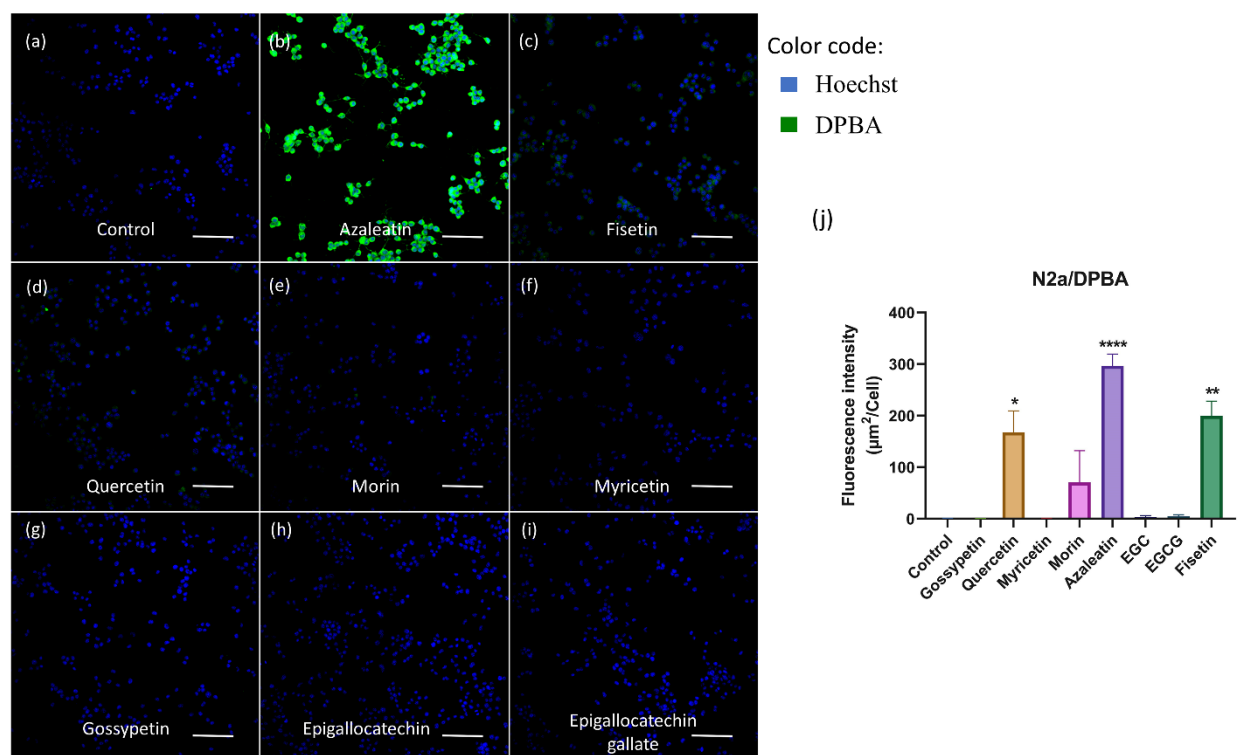
**Figure S3.** (a) A schematic presentation of the complexation of AlCl<sub>3</sub> with morin and the plausible quinonoid forms. (b) The plausible molecular structures of complexes formed on treatment of fisetin, quercetin, gossypetin, azaleatin, and myricetin with AlCl<sub>3</sub>.



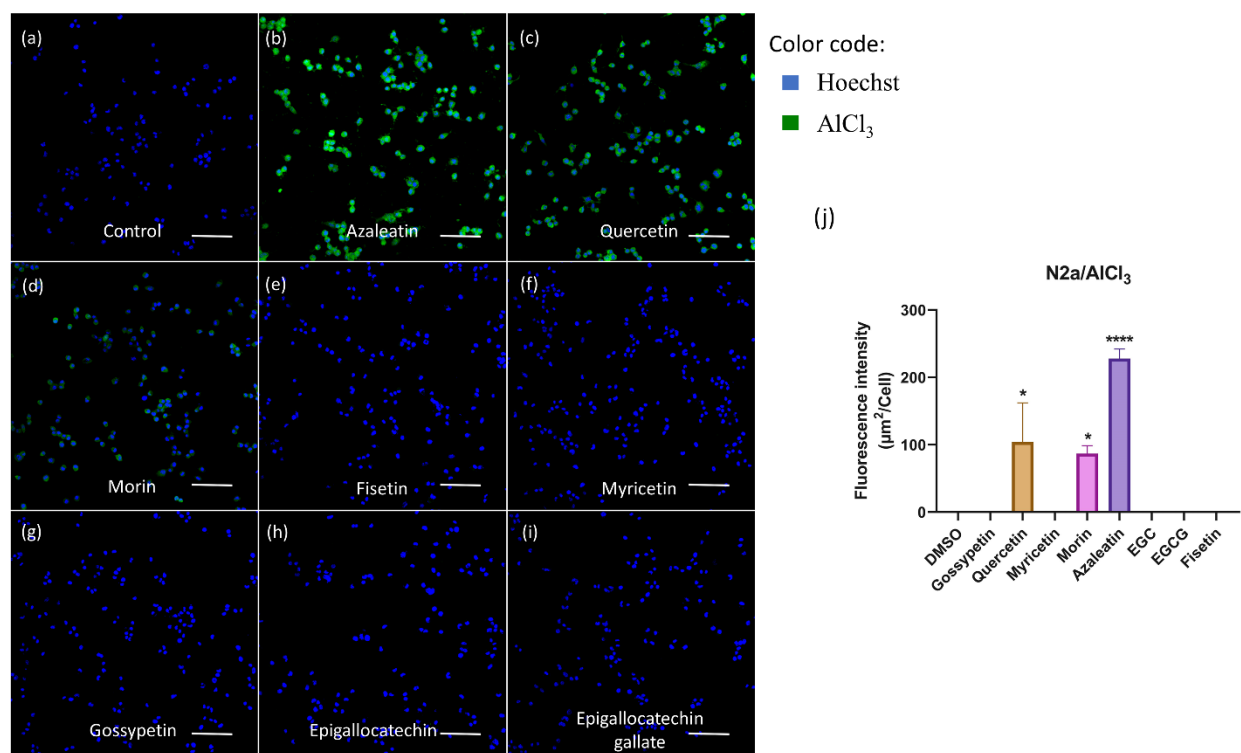
**Figure S4.** *In vitro* Investigation of fluorescence intensity of the flavonoids after chelation with  $\text{AlCl}_3$  (a-i) and a comparison of their relative intensity (j). The error bars represent the mean  $\pm$  SEM. \*\*\*\*P < 0.0001, \*\*\*P < 0.001, one-way ANOVA followed by Tukey's multiple comparisons test (j).



**Figure S5.** Fluorescence microscopic images of human neuroblastoma (SH-SY5Y) cells after flavonoid uptake followed by AlCl<sub>3</sub> treatment. Cells are treated with (a) DMSO (flavonoid absent, control), (b) azaleatin, (c) quercetin, (d) fisetin, (e) morin, (f) myricetin, (g) gossypetin, (h) epigallocatechin, and (i) epigallocatechin gallate. Flavonoid treatment (100 µM) is carried out for 1 h, while the enhancer AlCl<sub>3</sub> solution (0.01 M in water) is employed for 10 min. The scale bars represent 100 µm. Nucleus was stained using Hoechst. Among these, the azaleatin, quercetin, fisetin and morin treated cells showed fluorescence while it was not detectable in gossypetin, epigallocatechin, and epigallocatechin gallate treated cell. Panel (j) represents a comparison of fluorescence intensity exhibited by the flavonoid treated cells. The error bars represent the mean ± SEM. \*\*\*\*P < 0.0001, one-way ANOVA followed by Tukey's multiple comparisons test (j).

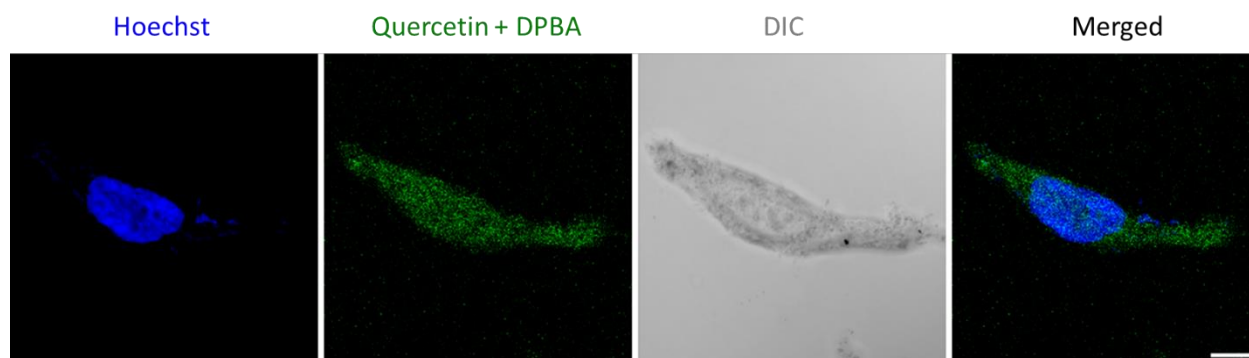


**Figure S6.** Fluorescence microscopic images of mouse neuroblastoma (N2a) cells after flavonoid uptake followed by DPBA treatment. Cells are treated with (a) DMSO (flavonoid absent, control), (b) azaleatin, (c) quercetin, (d) fisetin, (e) morin, (f) myricetin, (g) gossypetin, (h) epigallocatechin, and (i) epigallocatechin gallate. Flavonoid treatment (100  $\mu\text{M}$ ) is carried out for 1 h, while the enhancer DPBA solution (0.5% wt./v, in ethanol) is employed for 10 min. The scale bars represent 100  $\mu\text{m}$ . Nucleus was stained using Hoechst. Among these, the azaleatin, quercetin, fisetin and morin treated cells showed fluorescence while it was not detectable in gossypetin, epigallocatechin, and epigallocatechin gallate treated cell. Panel (j) represents a comparison of fluorescence intensity exhibited by the flavonoid treated cells. The error bars represent the mean  $\pm$  SEM. \*\*\*\* $P < 0.0001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , one-way ANOVA followed by Tukey's multiple comparisons test (j).



**Figure S7.** Fluorescence microscopic images of human neuroblastoma (N2a) cells after flavonoid uptake followed by  $AlCl_3$  treatment. Cells are treated with (a) DMSO (flavonoid absent, control), (b) azaleatin, (c) quercetin, (d) fisetin, (e) morin, (f) myricetin, (g) gossypetin, (h) epigallocatechin, and (i) epigallocatechin gallate. Flavonoid treatment (100  $\mu$ M) is carried out for 1 h, while the enhancer  $AlCl_3$  solution (0.01 M in water) is employed for 10 min. The scale bars represent 100  $\mu$ m. Nucleus was stained using Hoechst. Among these, the azaleatin, quercetin, fisetin and morin treated cells showed fluorescence while it was not detectable in gossypetin, epigallocatechin, and epigallocatechin gallate treated cell. Panel (j) represents a comparison of fluorescence intensity exhibited by the flavonoid treated cells. The error bars represent the mean  $\pm$  SEM. \*\*\*\*P < 0.0001, \*P < 0.05, one-way ANOVA followed by Tukey's multiple comparisons test (j).





**Figure S9.** A magnified image of quercetin treated SH-SY5Y single cell stained with DPBA showing the distribution of quercetin inside the cell. The scale bar represents 10  $\mu\text{m}$ .