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Fluorescence “off” and “on” signalling of esculetin in presence of copper and thiol: A possible implication in cellular thiol sensing

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\textbf{Fig. S1} UV-Visible absorption spectra of esculetin (50 \(\mu\text{M}\)) with Cu(II) (5-100 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM). Inset shows ratiometric change in absorbance of Cu(II) to Esculetin at 350 nm (a) and 389 nm (b).

\textbf{Fig. S2} Jobs plot of esculetin (50 \(\mu\text{M}\)) with Cu(II) (50 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM)

\textbf{Fig. S3} plot of log\([\text{F}_{\text{max}}/\text{F}]/\text{log(Cu(II))}\) versus log[Cu(II)] for titration of Cu(II) (10-50 \(\mu\text{M}\)) with esculetin (50 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM). \(\lambda_{\text{ex}} = 360\text{ nm, } E_{\text{ex}}/E_{\text{em}} \text{ Slit} = 2.5\text{ nm.}\)

\textbf{Fig. S4} Time course measurement of esculetin (50 \(\mu\text{M}\)) and Cu(II) (80 \(\mu\text{M}\)) with GSH (200 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM). \(\lambda_{\text{ex}} = 360\text{ nm, } \lambda_{\text{em}} = 466\text{ nm, } E_{\text{ex}}/E_{\text{em}} \text{ Slit} = 2.5\text{ nm.}\)

\textbf{Fig. S5} HRMS spectra of esculetin (500 \(\mu\text{M}\)) and Cu(II) (800 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM). ‘Esc’ refers to Esculetin

\textbf{Fig. S6} Cyclic voltamogramm of 800 \(\mu\text{M}\) Cu(II), 500 \(\mu\text{M}\) esculetin and mixture of the above two compounds in 0.1 M NaClO\textsubscript{4} used as supporting electrolyte.

\textbf{Fig. S7} UV-Visible absorption spectra of esculetin (50 \(\mu\text{M}\)), Cu(II) (80 \(\mu\text{M}\)) with GSH (10-200 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM). Inset shows change in absorbance in presence of GSH at 350 nm (a) and 389 nm (b).

\textbf{Fig. S8} UV-Visible absorption spectra esculetin (50 \(\mu\text{M}\)) in absence and presence of GSH (20-200 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM).

\textbf{Fig. S9} Fluorescence spectra of esculetin (50 \(\mu\text{M}\)) in absence and presence of GSH (20-200 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM). \(\lambda_{\text{ex}} = 360\text{ nm, } E_{\text{ex}}/E_{\text{em}} \text{ Slit} = 2.5\text{ nm.}\)

\textbf{Fig. S10} HRMS spectra of GSH (3 \(\mu\text{M}\)) in pH 7 phosphate buffer (10 mM).

\textbf{Fig. S11}. Relative fluorescence ‘on/off’ cycles of Cu(II)-esculetin by the subsequent addition of 200 \(\mu\text{M}\) of GSH/NEM. \(\lambda_{\text{ex}} = 360\text{ nm, } \lambda_{\text{em}} = 466\text{ nm, } E_{\text{ex}}/E_{\text{em}} \text{ Slit} = 2.5\text{ nm.}\)

\textbf{Fig. S12}. Cell viability study of CHO cells by MTT assay in presence of 25 \(\mu\text{M}\) esculetin, 40 \(\mu\text{M}\) Copper (II) and mixture of 25 \(\mu\text{M}\) esculetin & 40 \(\mu\text{M}\) Copper (II) ion. The results are presented as mean \(\pm\) SD, \(n = 2\). *p \(<\) 0.05 compared to control cells by T-test.
**Fig. S1** UV-Visible absorption spectra of esculetin (50 µM) with Cu(II) (5-100 µM) in pH 7 phosphate buffer (10 mM). Inset shows ratiometric change in absorbance of Cu(II) to Esculetin at 350 nm (a) and 389 nm (b).

**Fig. S2** Jobs plot of esculetin (50 µM) with Cu(II) (50 µM) in pH 7 phosphate buffer (10 mM).
Fig. S3 plot of log[(F-F₀)/(F_{max}-F)] versus log[Cu(II)] for titration of Cu(II) (10-50 µM) with esculetin (50 µM) in pH 7 phosphate buffer (10 mM). \( \lambda_{ex} = 360 \text{ nm} \), \( E_x/E_m \text{ Slit} = 2.5 \text{ nm} \).

Fig. S4 Time course measurement of esculetin (50 µM) and Cu(II) (80 µM) with GSH (200 µM) in pH 7 phosphate buffer (10 mM). \( \lambda_{ex} = 360 \text{ nm} \), \( \lambda_{em} = 466 \text{ nm} \), \( E_x/E_m \text{ Slit} = 2.5 \text{ nm} \).
**Fig. S5** HRMS spectra of esculetin (500 µM) and Cu(II) (800 µM) in pH 7 phosphate buffer (10 mM). ‘Esc’ refers to Esculetin

**Fig. S6** Cyclic voltammogram of 800 µM Cu(II), 500 µM esculetin and mixture of the above two compounds in 0.1 M NaClO₄ used as supporting electrolyte.
**Fig. S7** UV-Visible absorption spectra of esculetin (50 µM), Cu(II) (80 µM) with GSH (10-200 µM) in pH 7 phosphate buffer (10 mM). Inset shows change in absorbance in presence of GSH at 350 nm (a) and 389 nm (b).

**Fig. S8** UV-Visible absorption spectra esculetin (50 µM) in absence and presence of GSH (20-200 µM) in pH 7 phosphate buffer (10 mM).
Fig. S9 Fluorescence spectra of esculetin (50 µM) in absence and presence of GSH (20-200 µM) in pH 7 phosphate buffer (10 mM). $\lambda_{ex} = 360$ nm, $E_x/E_m$ Slit = 2.5 nm.

Fig. S10 HRMS spectra of GSH (3 mM) in pH 7 phosphate buffer (10 mM).
**Fig. S11.** Relative fluorescence 'on/off' cycles of Cu(II)-esculetin by the subsequent addition of 200 µM of GSH/NEM. $\lambda_{ex} = 360$ nm, $\lambda_{em} = 466$ nm, $E_x/E_m$ Slit = 2.5 nm.

**Fig. S12.** Cell viability study of CHO cells by MTT assay in presence of 25 µM esculetin, 40 µM Copper (II) and mixture of 25 µM esculetin & 40 µM Copper (II) ion. The results are presented as mean ± SD, n = 2. *p < 0.05 compared to control cells by T-test.