

Supporting information (Manuscript ID PP-ART-04-2018-000157)

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

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Fig. S1 UV-Visible absorption spectra of esculetin (50 μ M) with Cu(II) (5-100 μ 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).

Fig. S2 Jobs plot of esculetin (50 μ M) with Cu(II) (50 μ M) in pH 7 phosphate buffer (10 mM)

Fig. S3 plot of $\log[(F-F_0)/(F_{\max}-F)]$ versus $\log[\text{Cu(II)}]$ for titration of Cu(II) (10-50 μ M) with esculetin (50 μ M) in pH 7 phosphate buffer (10 mM). $\lambda_{\text{ex}} = 360$ nm, E_x/E_m Slit = 2.5 nm.

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

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

Fig. S6 Cyclic voltammogram of 800 μ M Cu(II), 500 μ M 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 μ M), Cu(II) (80 μ M) with GSH (10-200 μ 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).

Fig. S8 UV-Visible absorption spectra esculetin (50 μ M) in absence and presence of GSH (20-200 μ M) in pH 7 phosphate buffer (10 mM).

Fig. S9 Fluorescence spectra of esculetin (50 μ M) in absence and presence of GSH (20-200 μ M) in pH 7 phosphate buffer (10 mM). $\lambda_{\text{ex}} = 360$ 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 μ M of GSH/NEM. $\lambda_{\text{ex}} = 360$ nm, $\lambda_{\text{em}} = 466$ nm, E_x/E_m Slit = 2.5 nm.

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

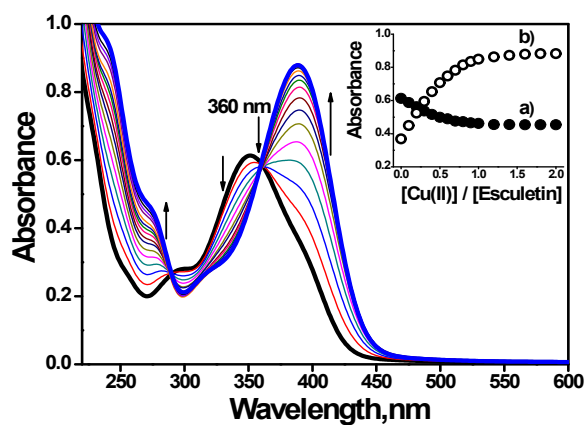


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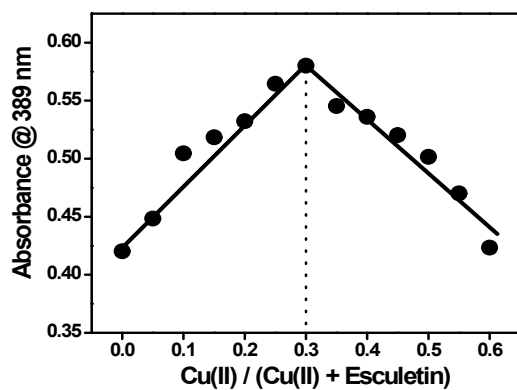


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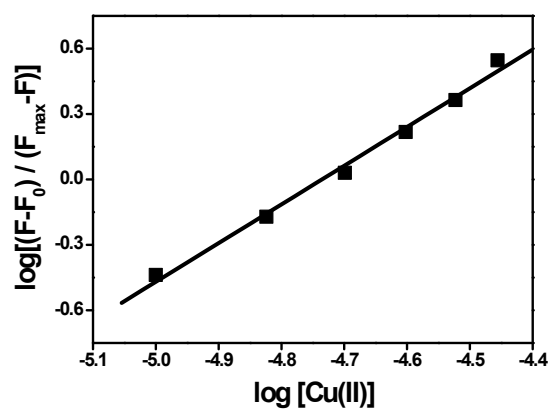


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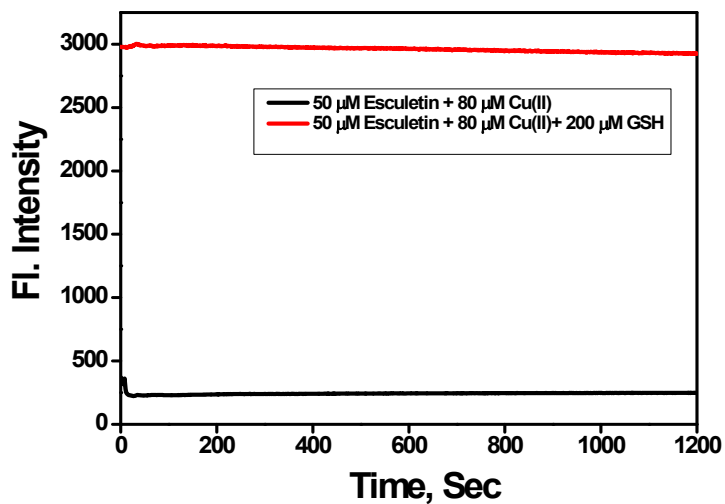


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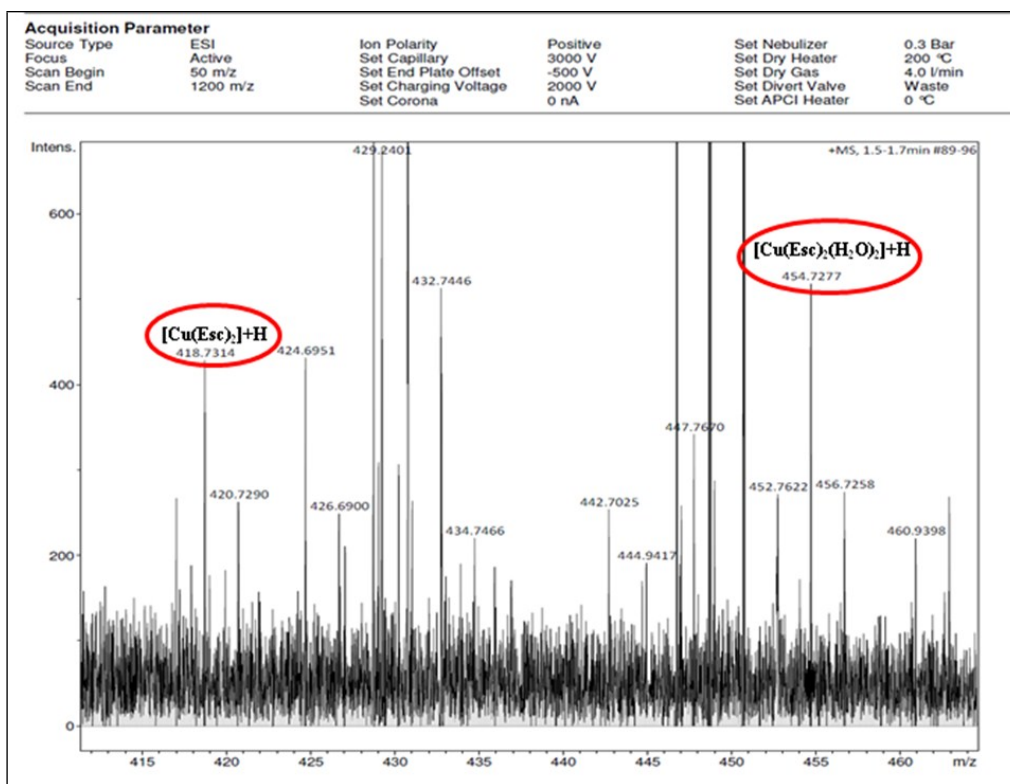


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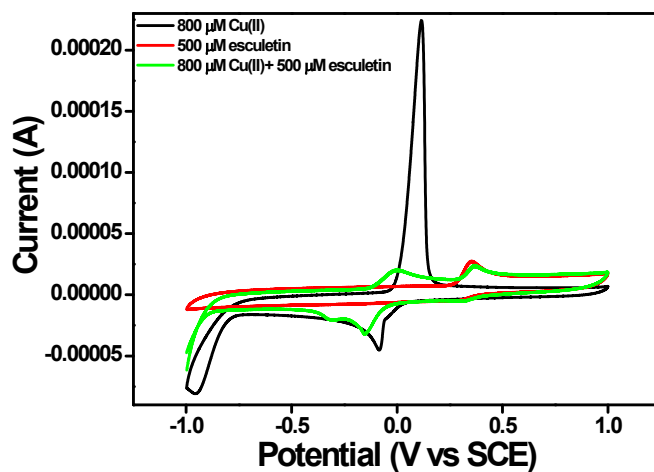


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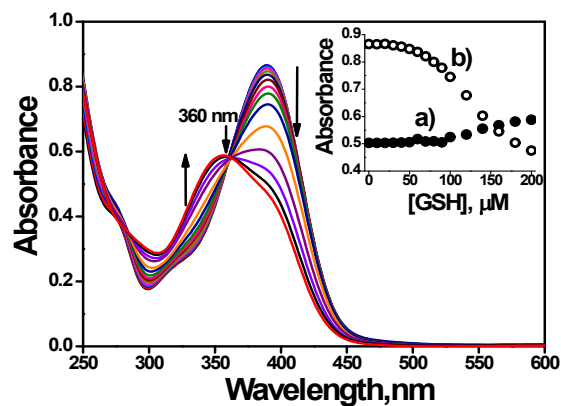


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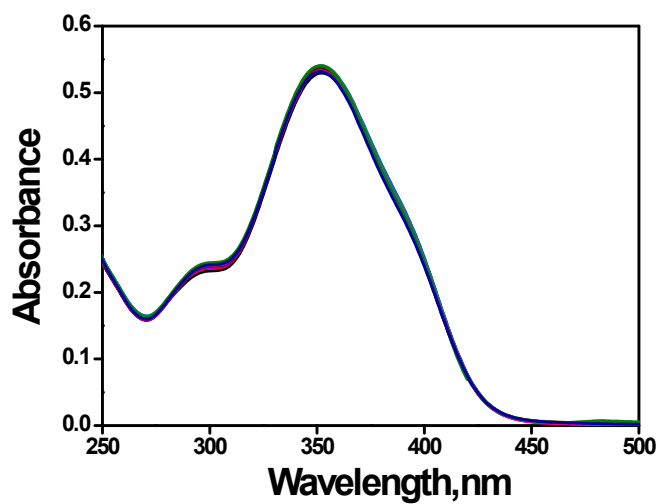


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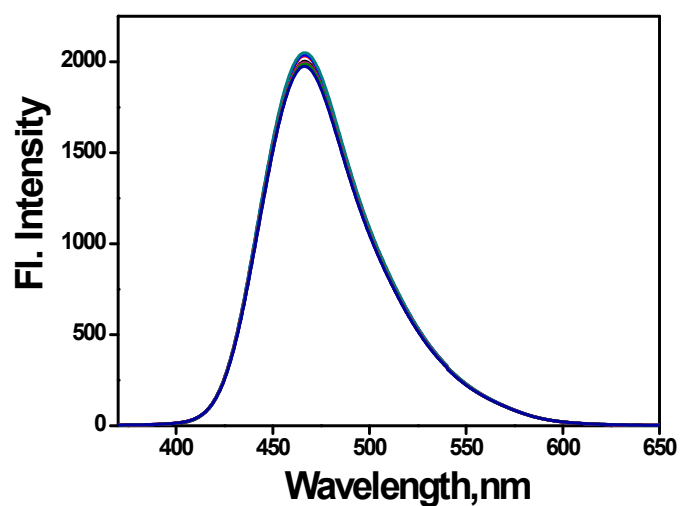


Fig. S9 Fluorescence spectra of esculetin (50 μ M) in absence and presence of GSH (20-200 μ M) in pH 7 phosphate buffer (10 mM). λ_{ex} = 360 nm, E_x/E_m Slit = 2.5 nm.

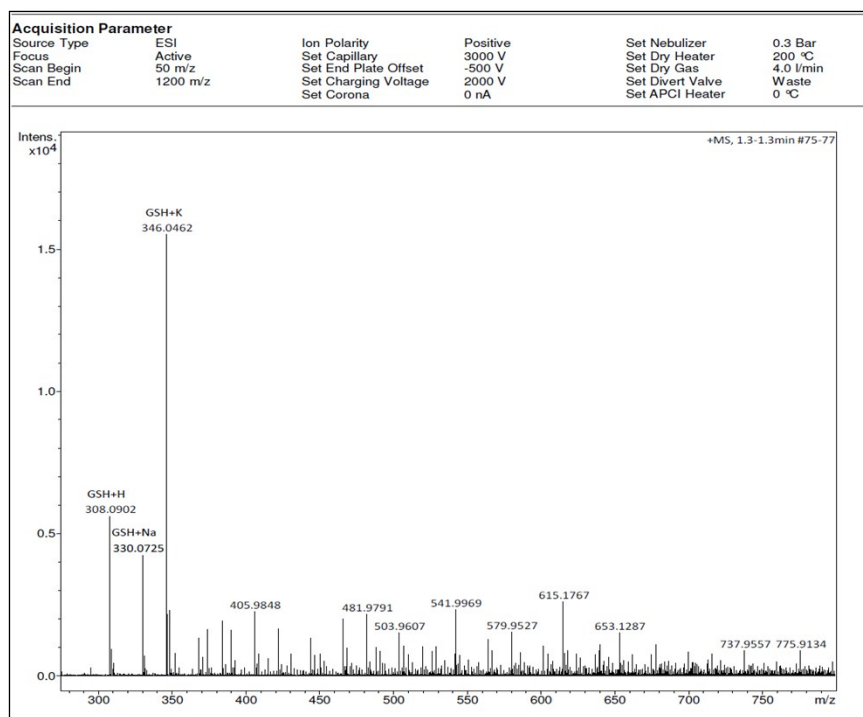


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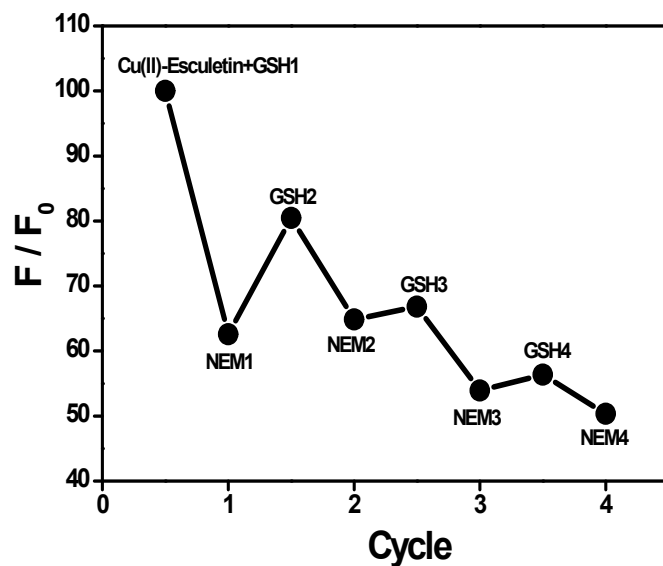


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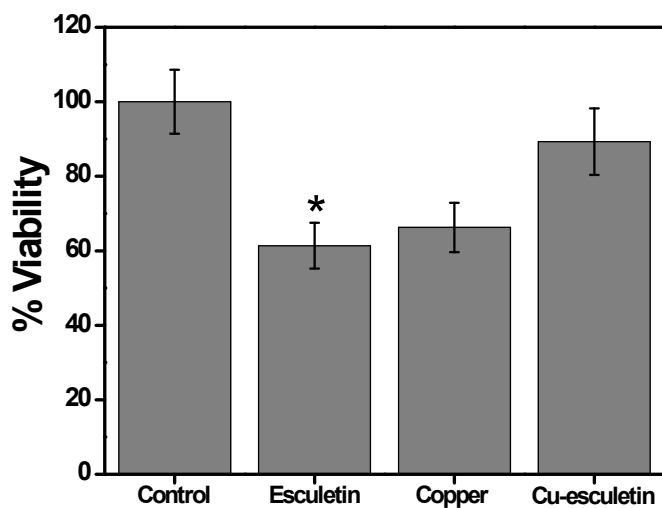


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