

SUPPLEMENTARY INFORMATION

**Fluorescence assay for the determination of glutathione based on novel ring-fused 2-pyridone derivative in dietary supplements**

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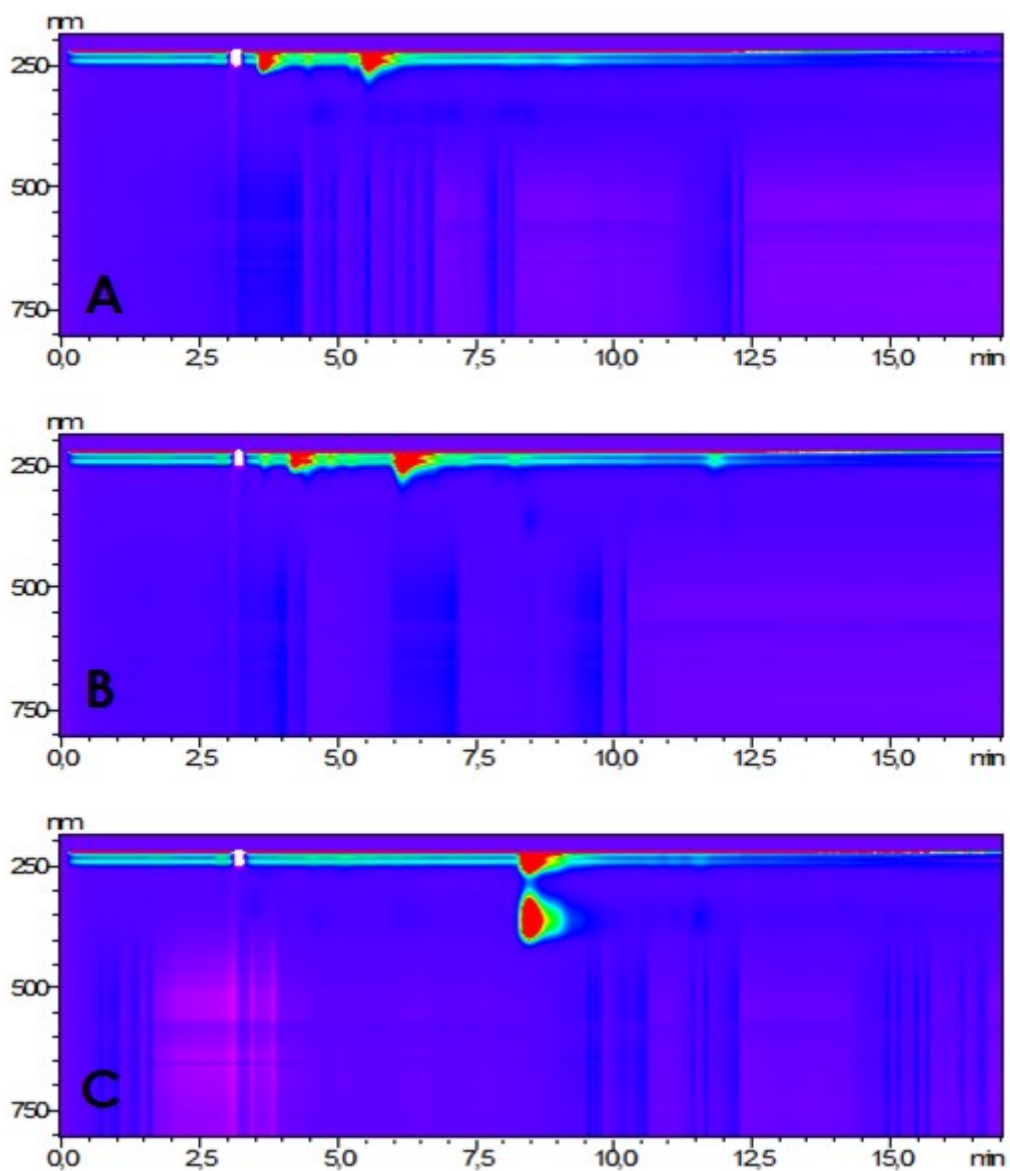
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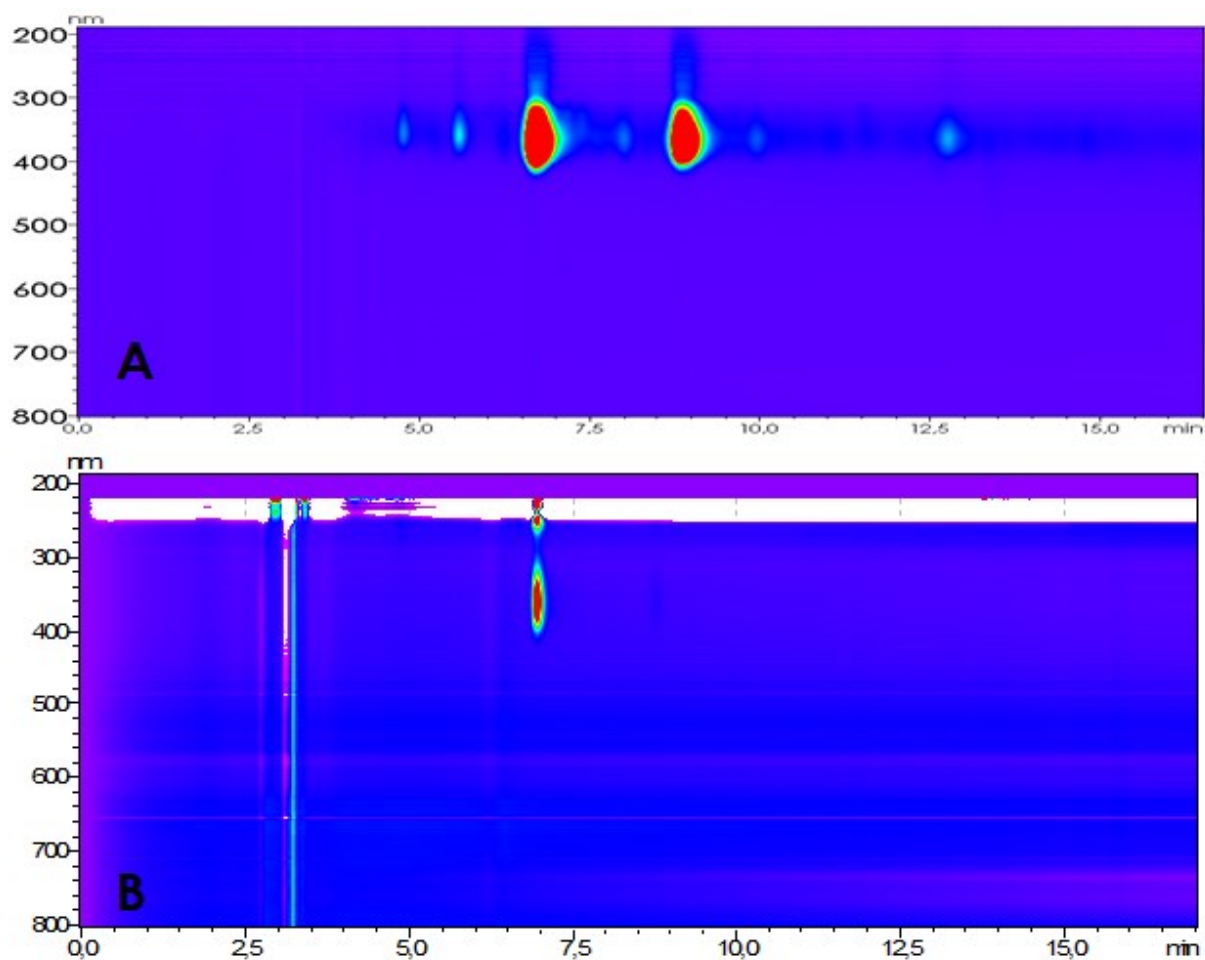
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**Table S1.** Comparison of peak area (2D chromatogram acquired at 350 nm) of the fluorescent fraction with retention time 9,3 minute following a various molar ratio of substrates.

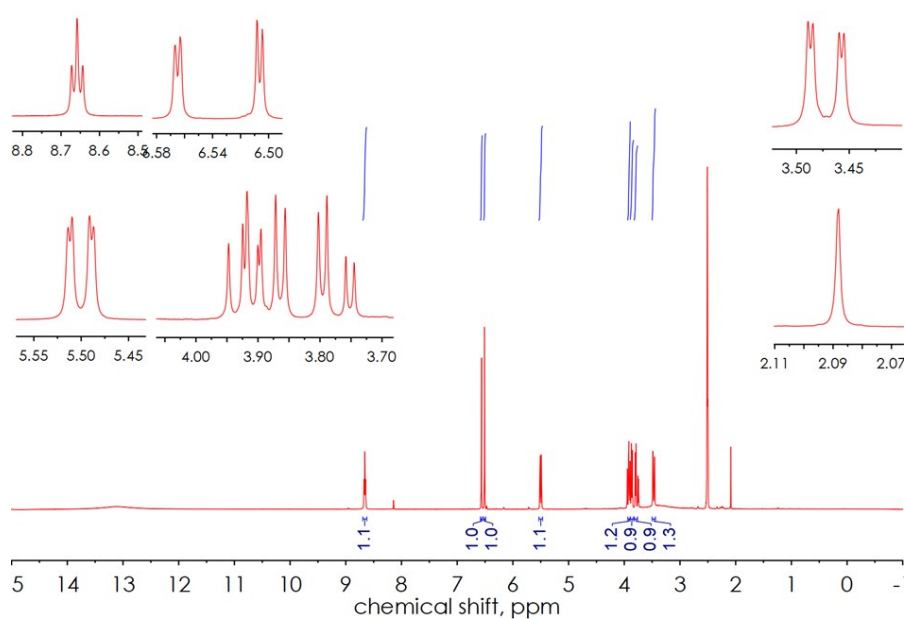
GSH:CA mol/mol	Peak area [mAU]
1:1	2703318
1:2	2490398
1:3	5132029
1:4	5127801
1:5	5632998
1:7,5	4813685
1:10	5148298
1:25	3198677
1:50	2639819



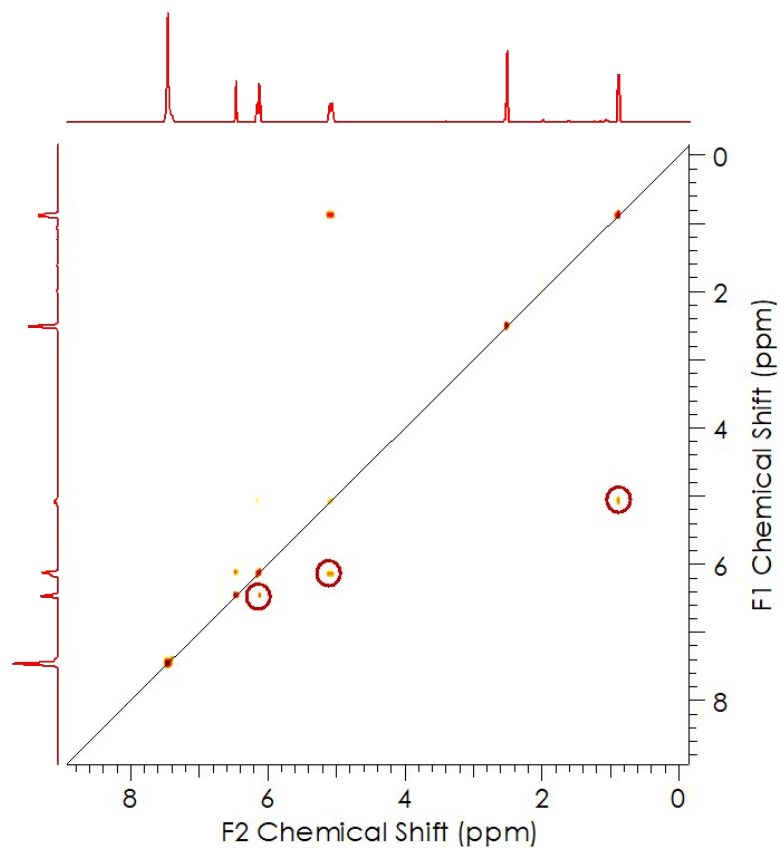
**Figure S1.** LC-DAD chromatograms of CA:glycine (A), CA:glutamic acid (B) and CA:L-cysteine (C) reaction mixtures.



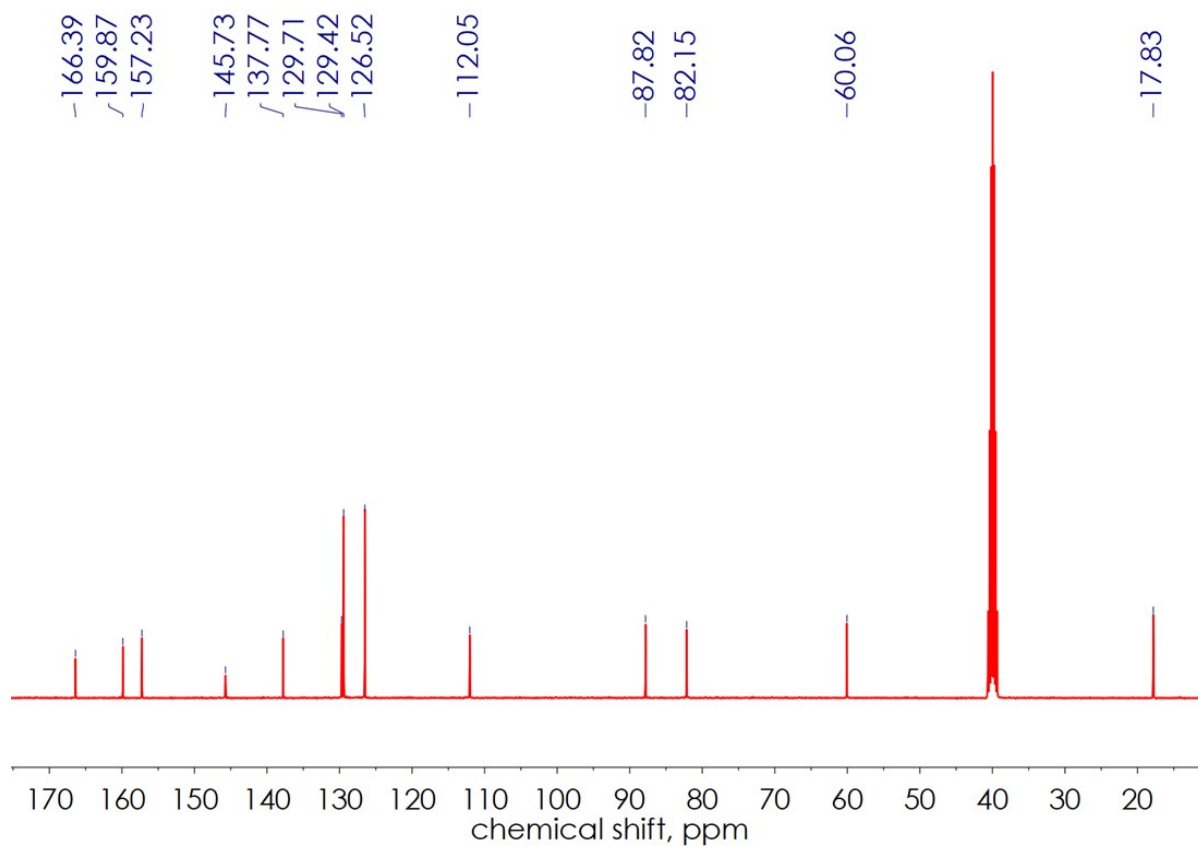
**Figure S2.** LC-UV-VIS chromatogram acquired during the preparative separation of CA:GSH reaction mixture – fraction eluted at 7.1 minutes was collected (A). LC-DAD chromatogram of purified fluorescent compound (B).



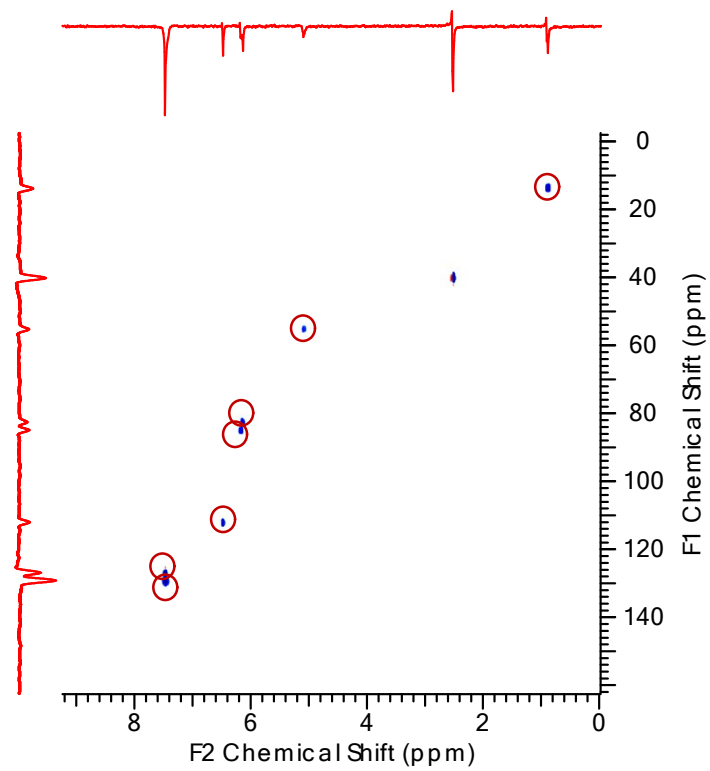
**Figure S3.**  $^1\text{H}$  NMR spectrum of the fluorophore.



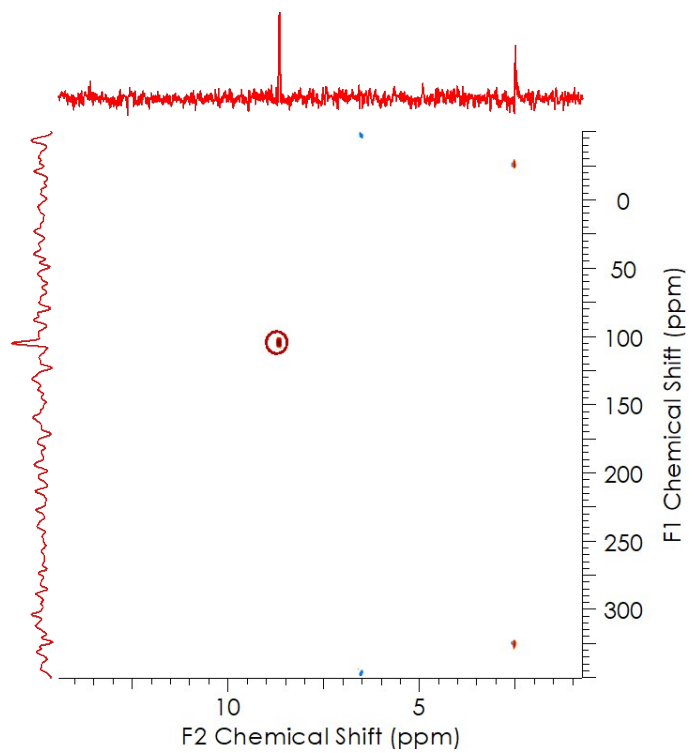
**Figure S4.** COSY  $^1\text{H}$ - $^1\text{H}$  spectrum of the fluorophore.



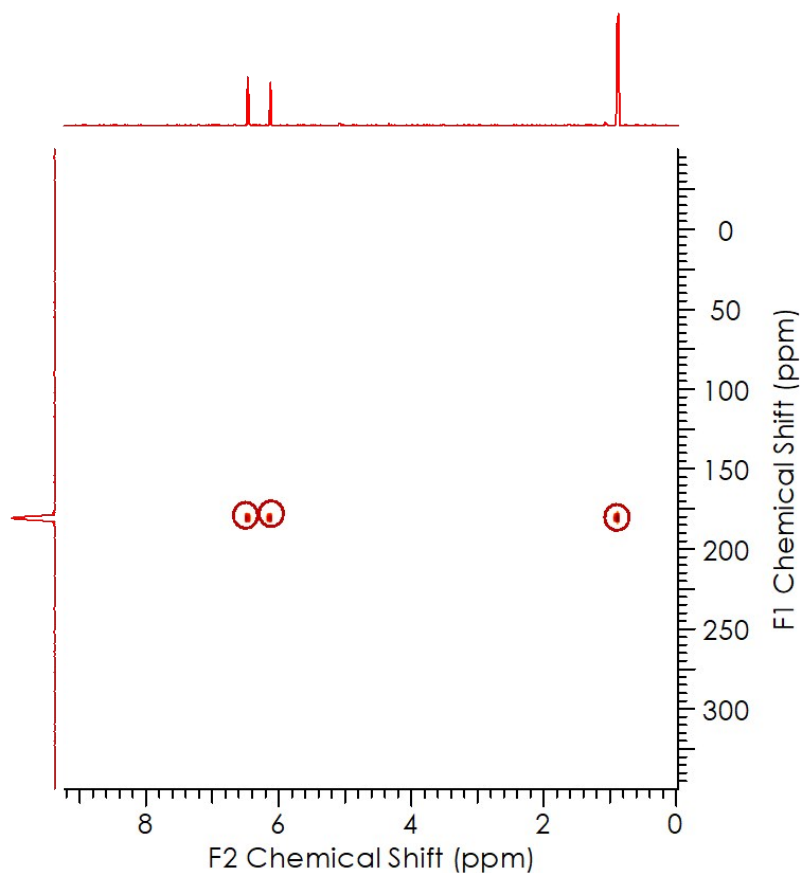
**Figure S5.**  $^{13}\text{C}$  NMR spectrum of the fluorophore.



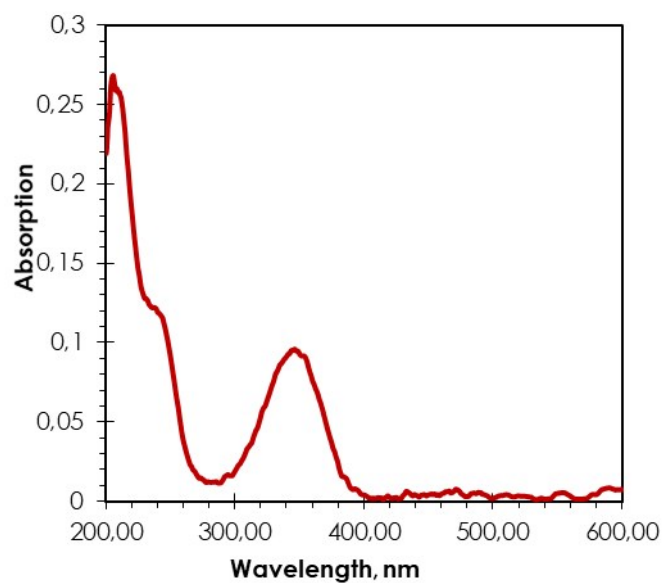
**Figure S6.** HSQC  $^{13}\text{C}$ - $^1\text{H}$  spectrum of the fluorophore.



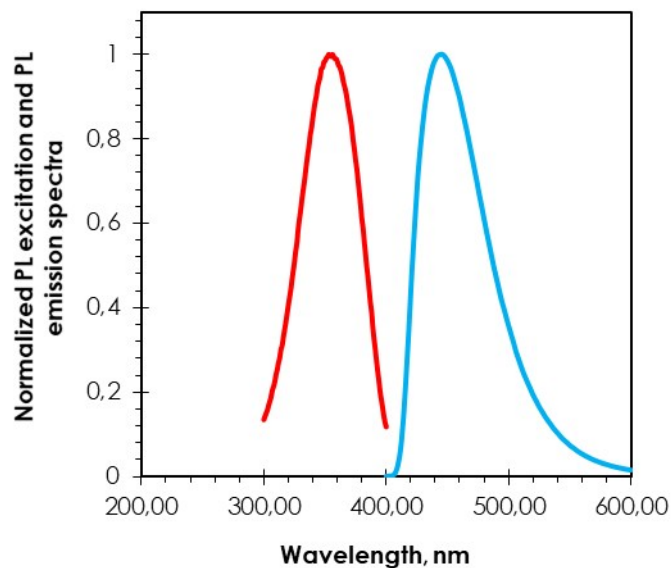
**Figure S7.** HSQC  $^{15}\text{N}$ - $^1\text{H}$  spectrum of the fluorophore.



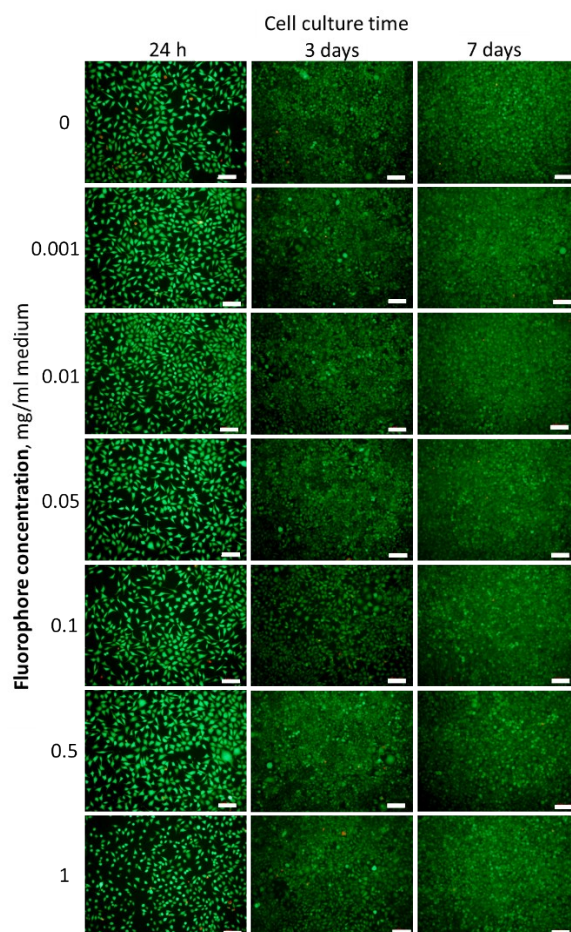
**Figure S8.** HMBC  $^{15}\text{N}$ - $^1\text{H}$  spectrum of the fluorophore.



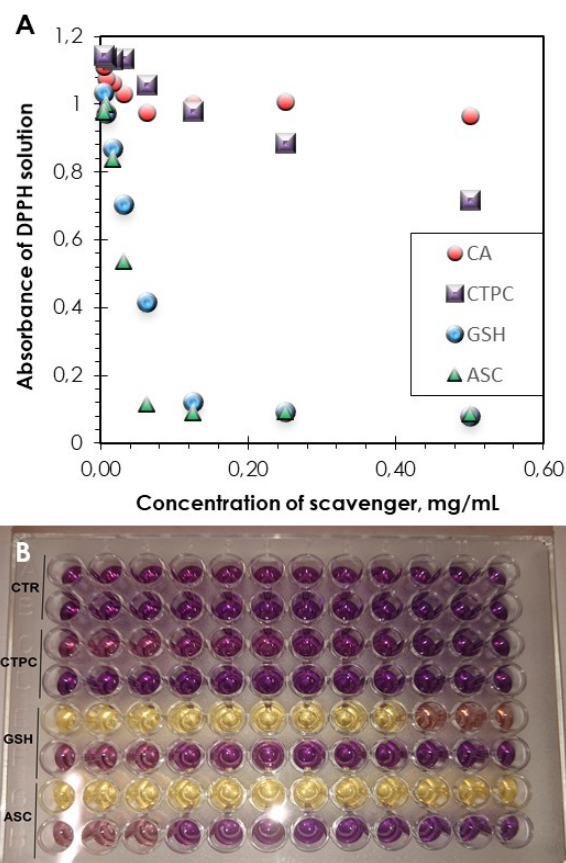
**Figure S9.** UV/VIS absorption spectrum of the aqueous solution of CTPC (0.05 mg/ml).



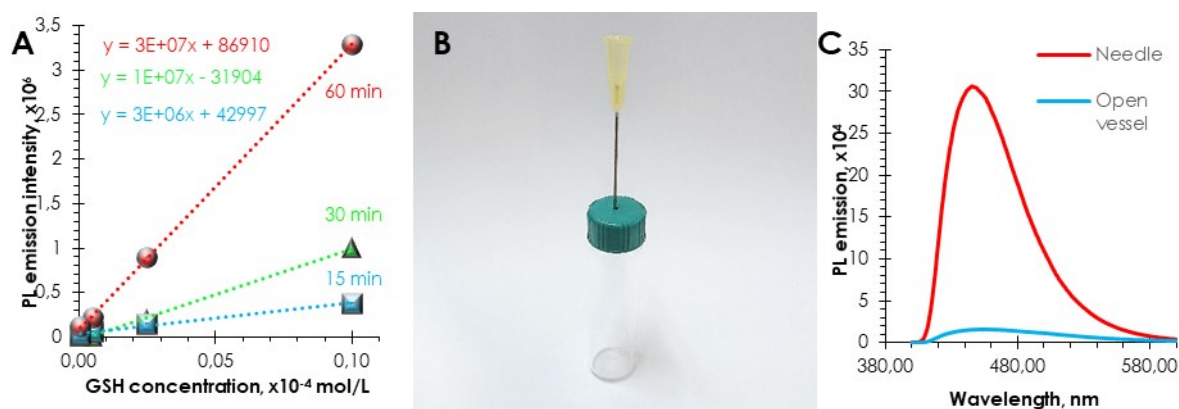
**Figure S10.** PL excitation and emission spectra of aqueous solution of CTPC (0.05 mg/ml). For PL excitation spectrum  $\lambda_{em}$  was set to 444 nm, while for PL emission spectrum spectra  $\lambda_{ex}$  was set to 355 nm, slits for both measurements were set to 5 nm both for excitation and emission.



**Figure S11.** Live-dead (calcein-AM and propidium iodide) staining of L929 cells cultured for 24 h, 3 and 7 days in control medium (0 mg/ml) and medium supplemented with fluorophore at concentration of 0.001, 0.01, 0.05, 0.5 and 1 mg/ml medium, scale bar = 100  $\mu$ m; no significant differences between samples on the same day of culture according to ANOVA.

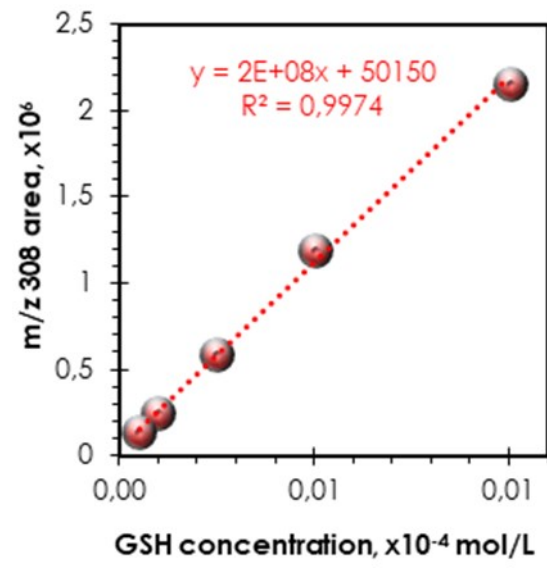


**Figure S12.** Antioxidative activity of CTPC in comparison to CA, GSH, and AA (A) and the photography of samples of DPPH solution treated with CA, GSH, and AA (B).



**Figure S13.** Comparison of PL emission of GSH derivatization reactions at various time intervals (A). Photography of the vials and cups used for the GSH derivatization protocol (B). Comparison of PL emission of GSH derivatization reactions conducted in open vessels and with controlled-release cups (C).





**Figure S14.** LC-ESI-MS calibration curve for GSH determination.