# Supplementary Material

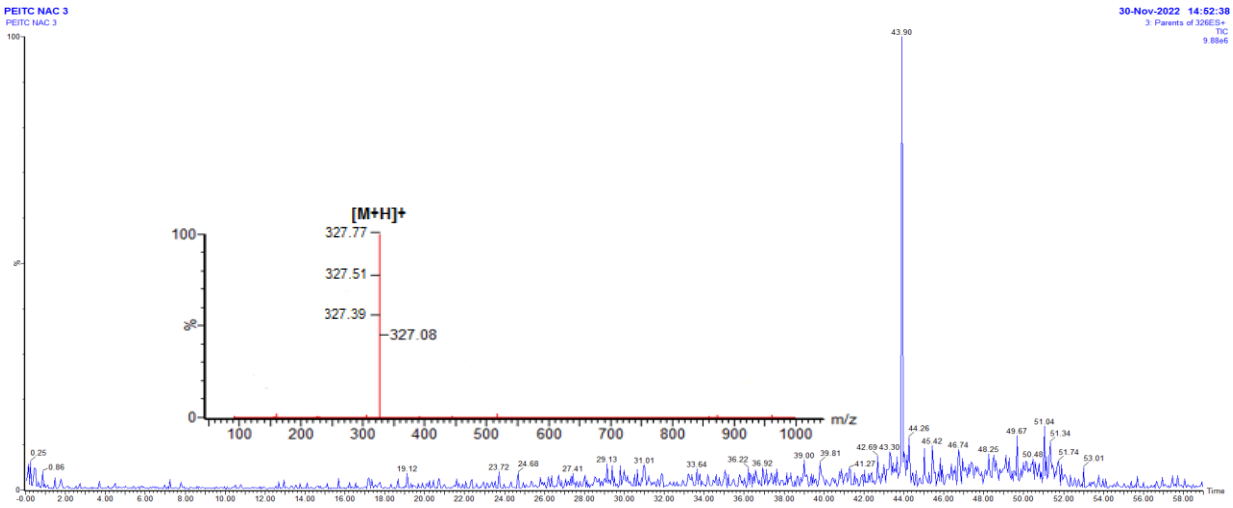
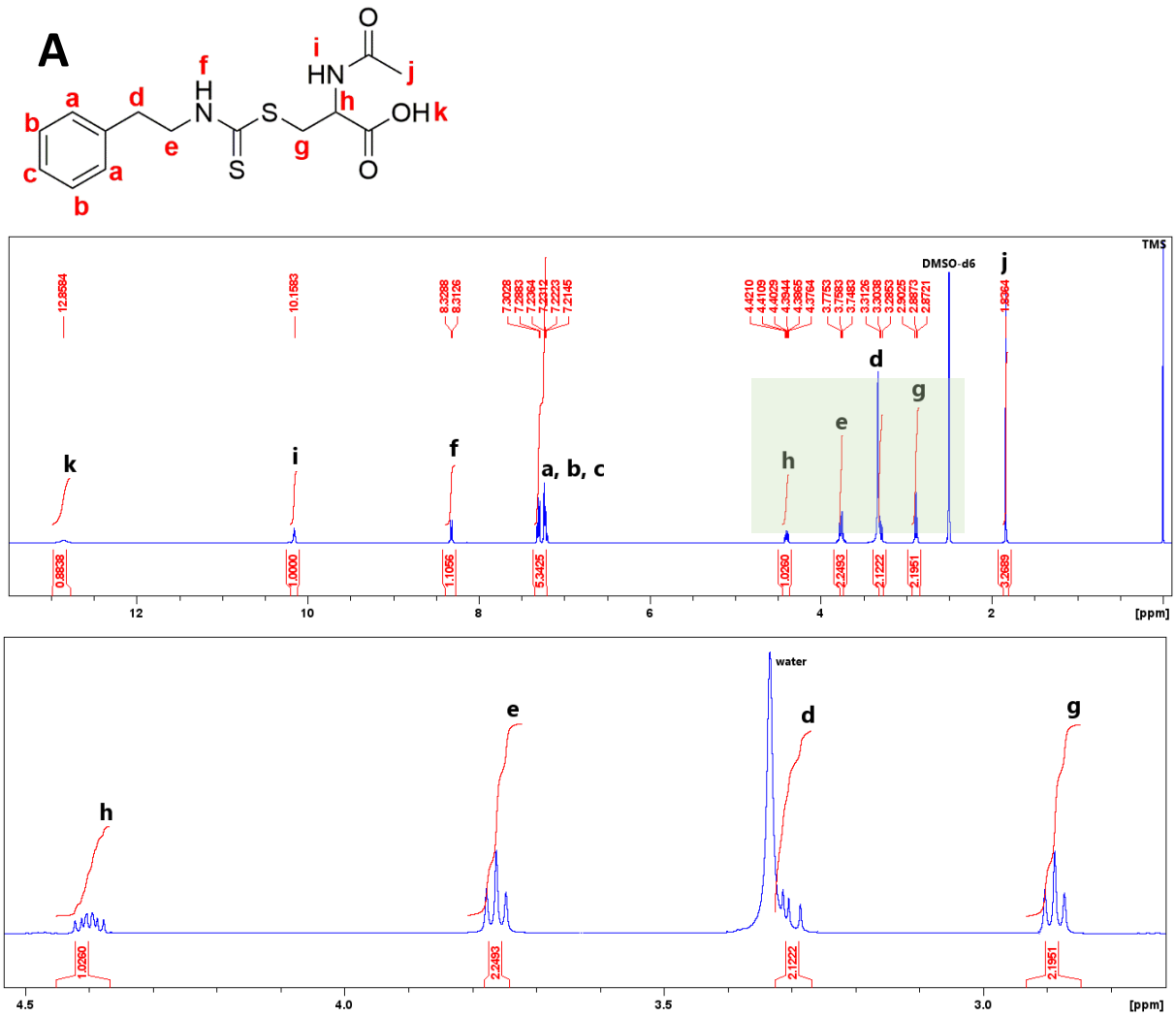



**Figure S1**: Mass spectrum of the synthesised PEITC-NAC conjugate.



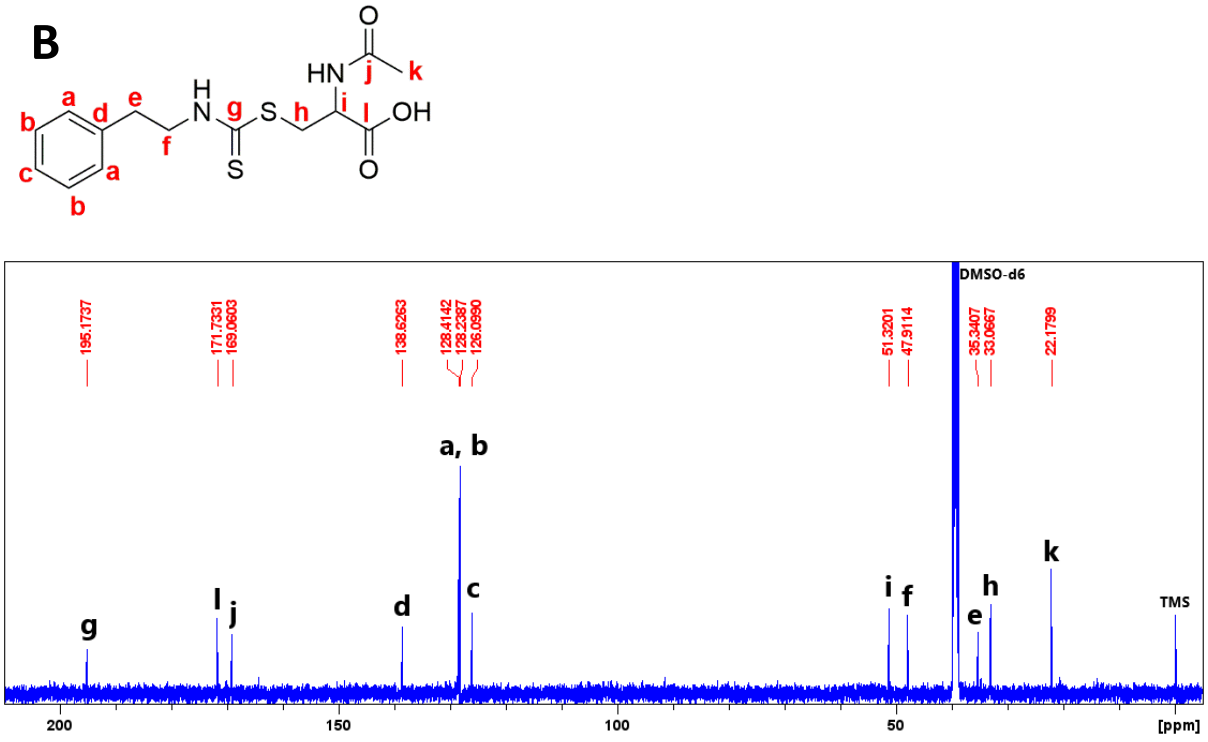



**Figure S2**: $^{1}$H-NMR **(A)** and $^{13}$C-NMR **(B)** spectra of *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione at 500 MHz and 100 MHz in DMSO-d$_6$ respectively.

**Table S1**: Multiple reaction monitoring conditions for *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione in UPLC-MS/MS analysis

| Compound | Chemical formula | MW | $[M-H]^+$ (m/z) | $MS^2$ fragments (m/z) | Cone voltage (V) | Collision energy (eV) | Retention time ($R_t$) (min) |
|---|---|---|---|---|---|---|---|
| *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione | $C_{14}H_{18}N_2O_3S_2$ | 326.43 | 327.77 | 197.42 | 15 | 23 | 43.9 |

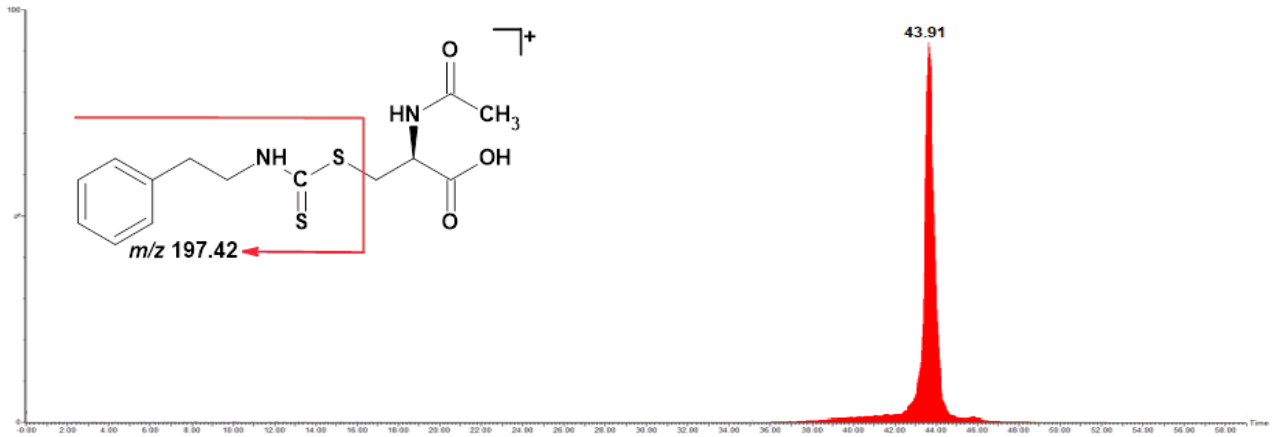


**Figure S3:** Extracted UPLC- ESI-(+)-MS/MS chromatogram of *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione.

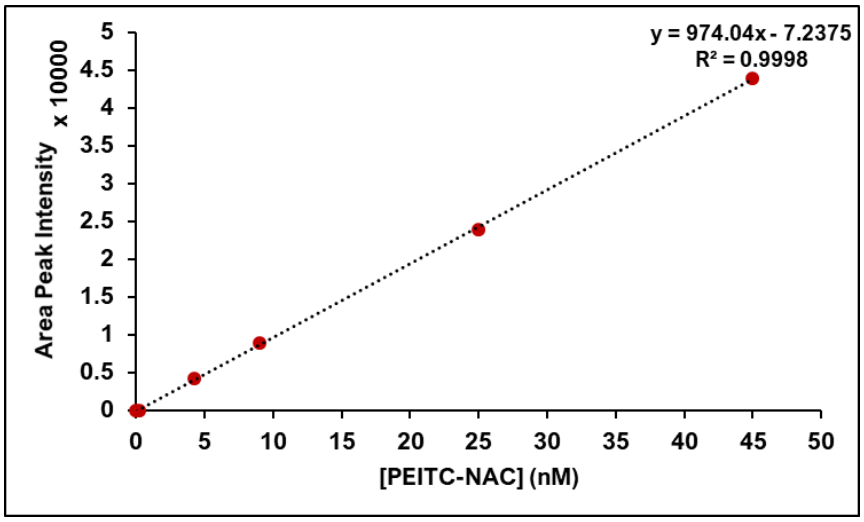



**Figure S4**: Calibration curve of *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione standard at various concentrations (0.1-45 nM) used for the determination of the mercapturic acid end-product in intracellular space and culture medium.

**Table S2**: The limit of detection (LOD), quantification (LOQ), linearity, precision and accuracy results for *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione. The calibration equations represent the peak area as a function of concentration in nM. The intra- and inter- day experimental data have been collected over a six days experiment. The % recovery data represent means of three independed experiments.

| **Compound** | **Linear range (nM)** | ***LoD* (nM)** | ***LoQ* (nM)** | **Calibration equation[a]** | **Correlation coefficient ($r^2$)** | **%RSD** | | **%REC[d]** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | **(intra-day)[b]** | **(inter-day)[c]** | |
| *N*α-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-glutathione | 0.21-45 | 0.21 | 6.93 | y = 974.04x-7.235 | 0.9998 | 0.96 | 2.56 | 98.8 |

[a] Chromatographic peak area (y) as a function of nM concentration (x)

[b] Values are means of intra-day assays (*n*=6)

[c] Values are means of inter-day assays (*n*=6)

[d] (*n*=3)

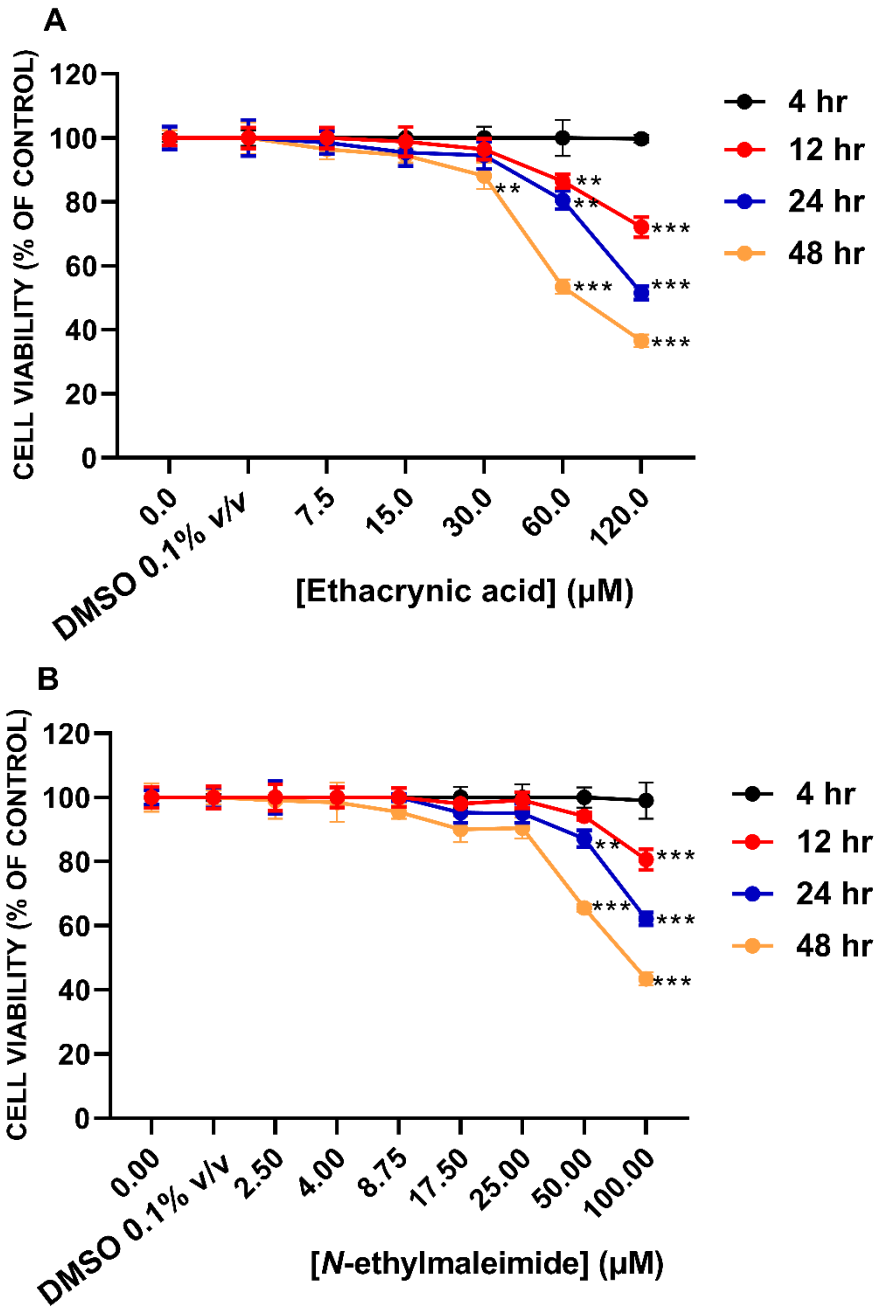



**Figure S5**: Cytotoxicity profiles of A375 cells subjected to either ethacrynic acid (0-120 μM) (**A**) or N-ethylmaleimide (0- 100 μM) (**B**) over 4-48 hr of exposure. Data are expressed as means ± SEM of three independed experiments. *n.d*. represents data not determined. Statistical significance is indicated by **at $p$<0.01, ***at $p$<0.001 relative to corresponding controls (DMSO 0.1% *v*/*v*).

**Table S3**: Calculated EC50 values for ethacrynic acid and *N*-ethylmaleimide, in all cell lines, at different time point of exposure by utilizing an online $EC_{50}$ calculator platform (Very Simple IC50 Tool Kit, available online: http://www.ic50.tk/ (accessed on 14 April 2023).

| Time (hr) | Ethacrynic acid | *N*-ethylmaleimide |
|---|---|---|
| | EC50 (μM) | |
| 4 | n.d. | n.d. |
| 12 | n.d. | n.d. |
| 24 | 114.98±4.21 | n.d. |
| 48 | 55.48±1.25 | 83.56±2.64 |