# Electronic Supplementary Information

## UNDERSTANDING THE METABOLISM OF THE ANTICANCER DRUG TRIAPINE: ELECTROCHEMICAL OXIDATION, IN VITRO AND IN VIVO ANALYSIS USING LC-HRMS

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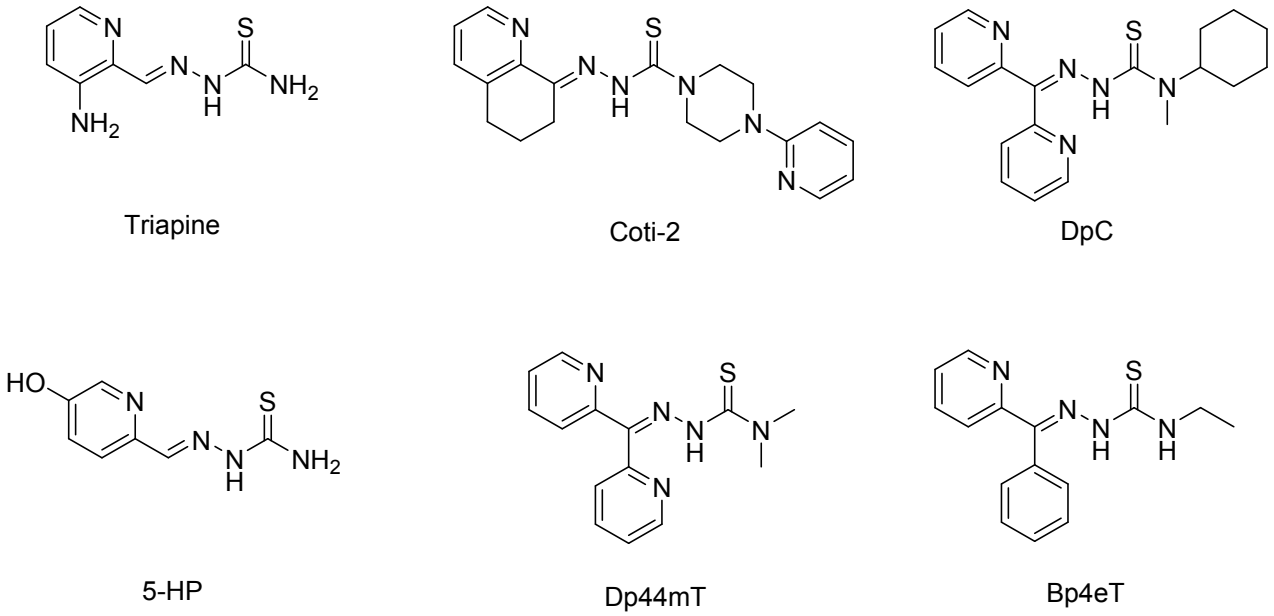
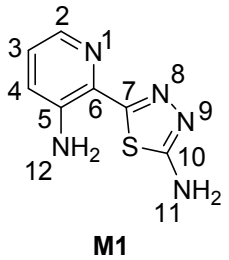



**Scheme S1.** Molecular structures of Triapine, Coti-2, DpC, 5-HP, Dp44mT and Bp4eT.




**Scheme S2.** Assignment of NMR data of M1.

$^{1}$H NMR (DMSO-$d_6$, 700.40 MHz, 298.2 K): $\delta$ 7.82 (dd, $^{2}J$ = 4.4 Hz, $^{3}J$ = 1.4 Hz, 1H, $H_{py\text{-}2}$), 7.36 (s, 2H, $H_{11}$), 7.19 (dd, $^{2}J$ = 8.3 Hz, $^{3}J$ = 1.4 Hz, 1H, $H_{py\text{-}4}$), 7.11 (dd, $^{2}J$ = 4.4 Hz, $^{2}J$ = 8.3 Hz, 1H, $H_{py\text{-}3}$), 6.77 (s, 2H, $H_{12}$) ppm. $^{13}$C NMR (DMSO-$d_6$, 176.13 MHz, 298.2 K): $\delta$ = 168.2 ($C_{10}$), 162.2 ($C_7$), 142.2 ($C_5$), 136.8 ($C_2$), 130.7 ($C_6$), 124.2 ($C_3$), 122.4 ($C_4$) ppm. $^{15}$N NMR (DMSO-$d_6$, 70.98 MHz, 298.2 K): $\delta$ = 312.4 ($N_1$), 303.3 ($N_9$), 66.8 ($N_{11}$, $J$ = 89.0 Hz), 65.3 ($N_{12}$, $J$ = 88.8 Hz) ppm ($N_8$ could not be observed because of the lack of a proton coupling).

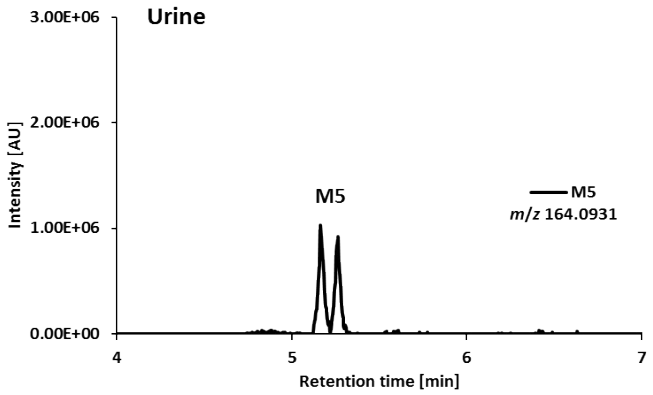



**Figure S1.** LC/ESI-HRMS analysis of the amidrazone metabolite (*m/z* 164.0931) of Triapine in urine upon oxidative desulfuration.

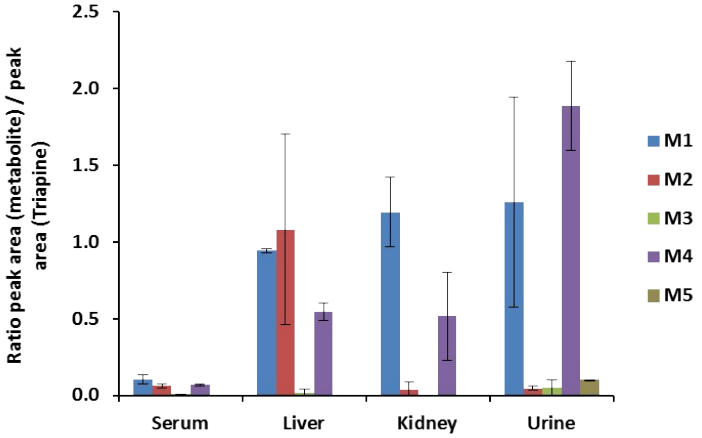



**Figure S2.** The ratio of the peak area of the different metabolites relative to the peak area of Triapine in serum, liver, kidney and urine *in vivo* samples 15 min after Triapine treatment.

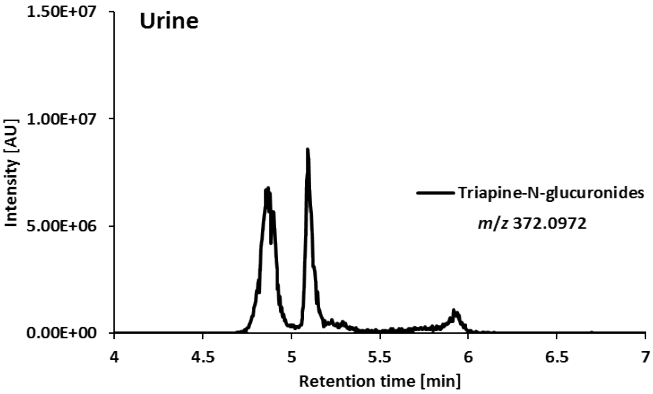



**Figure S3.** LC/ESI-HRMS analysis of *N*-glucuronides of Triapine (*m/z* 372.0972) in urine sample.

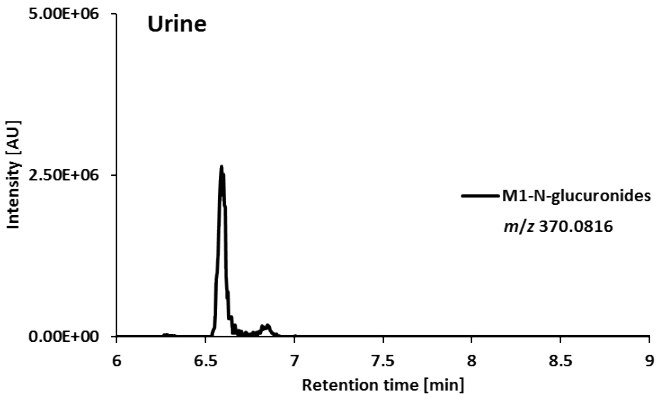



**Figure S4.** LC/ESI-HRMS analysis of *N*-glucuronides of M1 (*m/z* 370.0816) in urine sample.

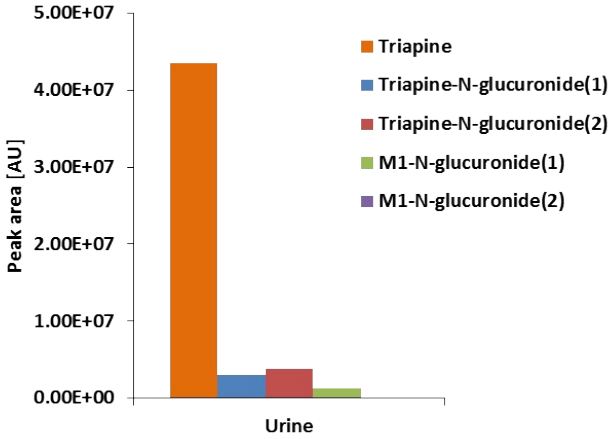



**Figure S5.** Peak areas of *N*-glucuronides in comparison to Triapine in urine* 15 min after *in vivo* Triapine treatment.

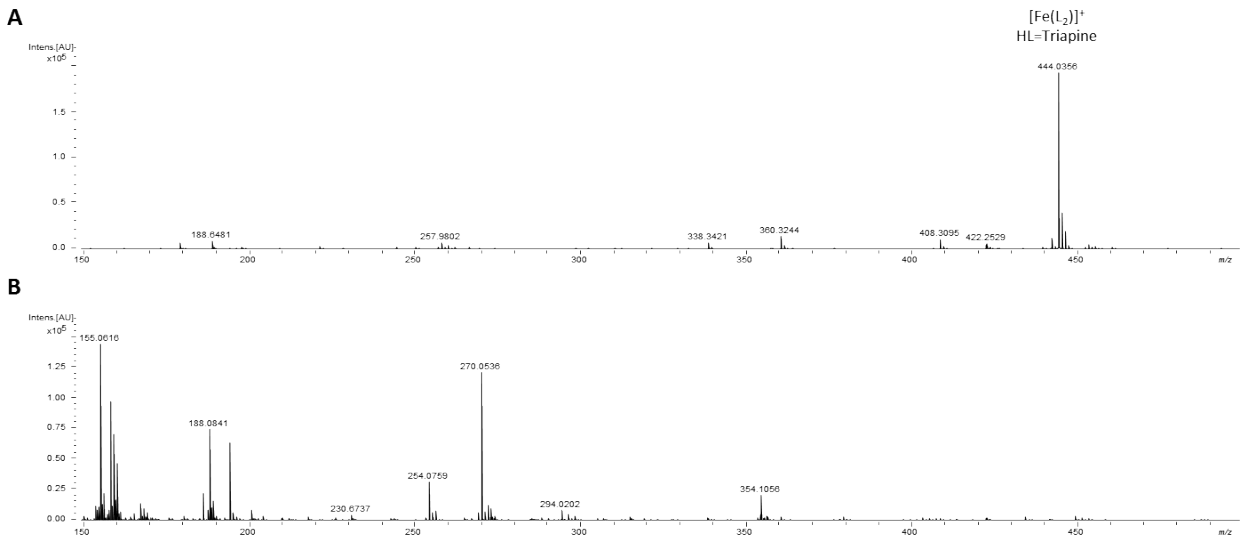



**Figure S6.** The HRMS spectra obtained after co-incubations of A) Triapine and B) M1 with iron(III) nitrate.

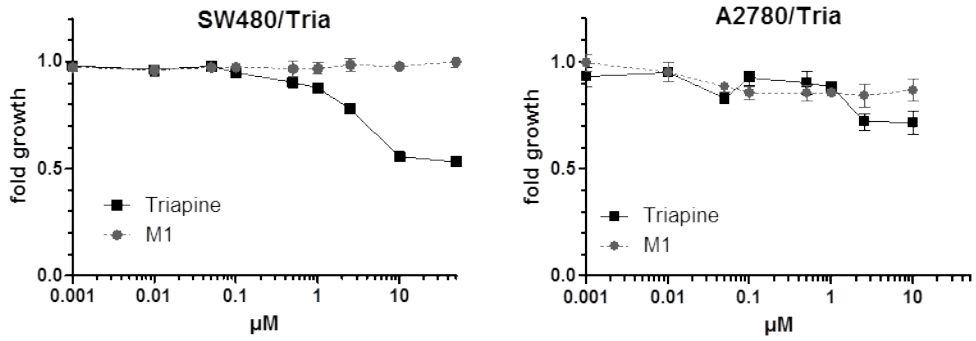



**Figure S7.** Cytotoxicity of Triapine compared to M1 in the Triapine resistant subclones SW480/Tria and A2780/Tria after 72 h treatment. Viability was determined using MTT assay. The values given are the mean ± the standard deviation of triplicates from one representative experiment out of two.