A new mechanism of action for the anticancer drug mitomycin C: Mechanism-based inhibition of thioredoxin reductase.

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Supporting Information

Figure S1. *pH-dependence of the inactivation of TrxR by MMC*. TrxR (100 nM) was incubated with MMC (60 µM) and NADPH (200 µM) in 100 mM phosphate buffer at pH values as indicated in inset. TrxR activity was measured at time intervals using the DTNB assay

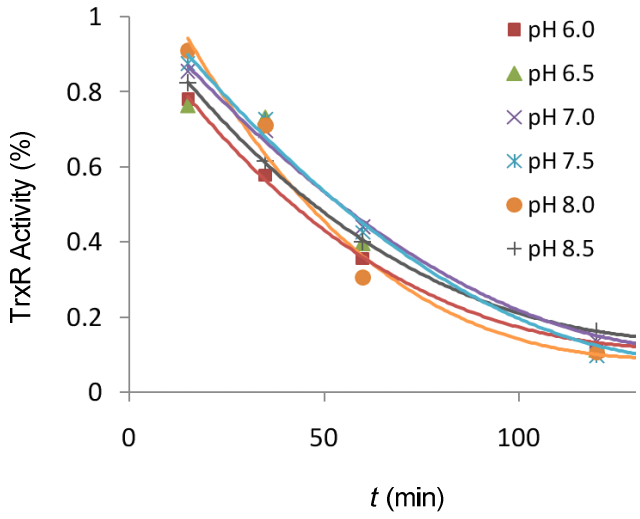


Figure S2. *MMC is not a substrate for TrxR*. The decrease in the absorbance at 340 nm was monitored in incubation mixtures containing the components indicated in inset. Concentrations were: 30 µM MMA or MMC, 200 µM NADPH, 25 nM TrxR. The buffer was TE pH 7.5

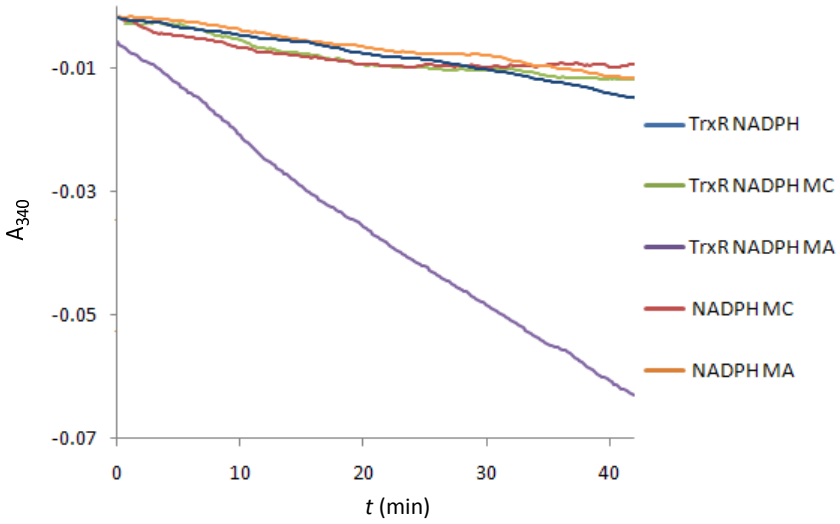


Figure S3. *MMC is not a substrate for TrxR*. The formation of mitosenes derived from the reductive activation of MMC was analyzed by measuring the increase of absorbance at 550 nM. Reaction mixtures contained the components indicated in inset. Concentrations were: 100 mM MMC, 200 mM NADPH, 50 nM TrxR. Additional incubations containing 200 mM GSH or ethyl xanthate were included to prevent inactivation of the enzyme. Positive controls were performed using DTT as reducing agent at concentrations as shown.

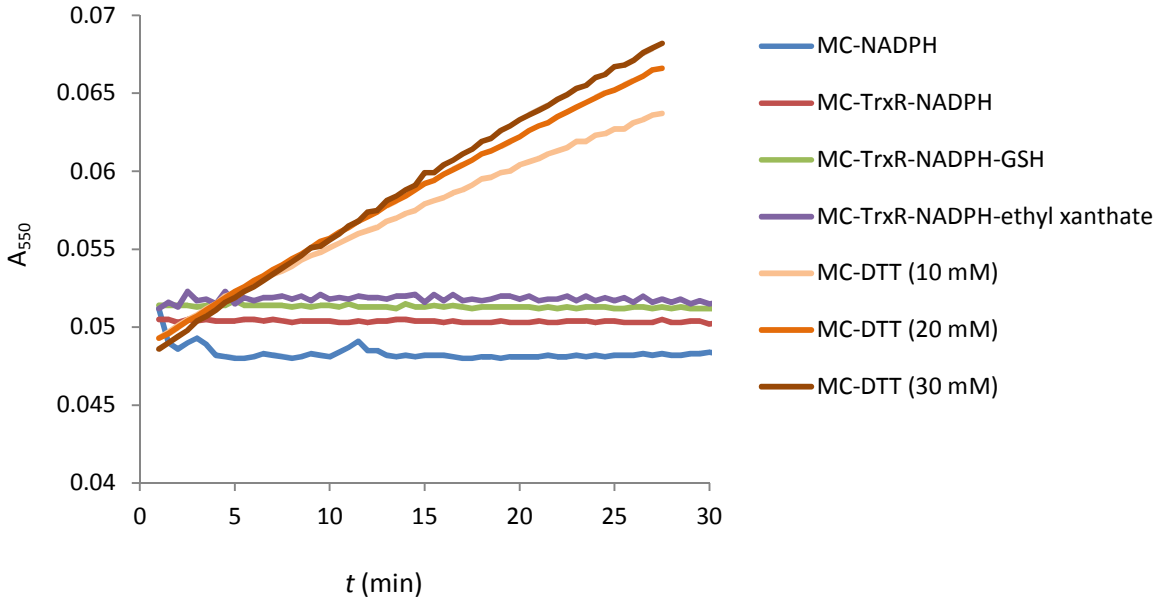


Figure S4. *MMC is not converted to mitosenes by TrxR-NADPH* . LC/MS analysis of a reaction containing 300 nM TrxR, 20 µM MMC, 100 µM NADPH in TE buffer pH 7.5 after 48 h. (a) Chromatogram at 315 nm, the absorbance maximum for mitosenes derived from MMC. (b) MS and UV spectra showing that the two peks observed in the chromatogram correspond to MMC. (c) Chromatogram (315 nm) from the LC/MS analysis of a reaction of chemically activated MMC showing the formation of 2,7-DAM and 1-hydroxymitosenes, and that these peaks elute with different retention times from MMC. Note that the molar extinction coefficient at 315 nm is much higher for mitosenes (10500 $M^{-1}cm^{-1}$) than that for MMC (c.a. 2000 $M^{-1}cm^{-1}$).

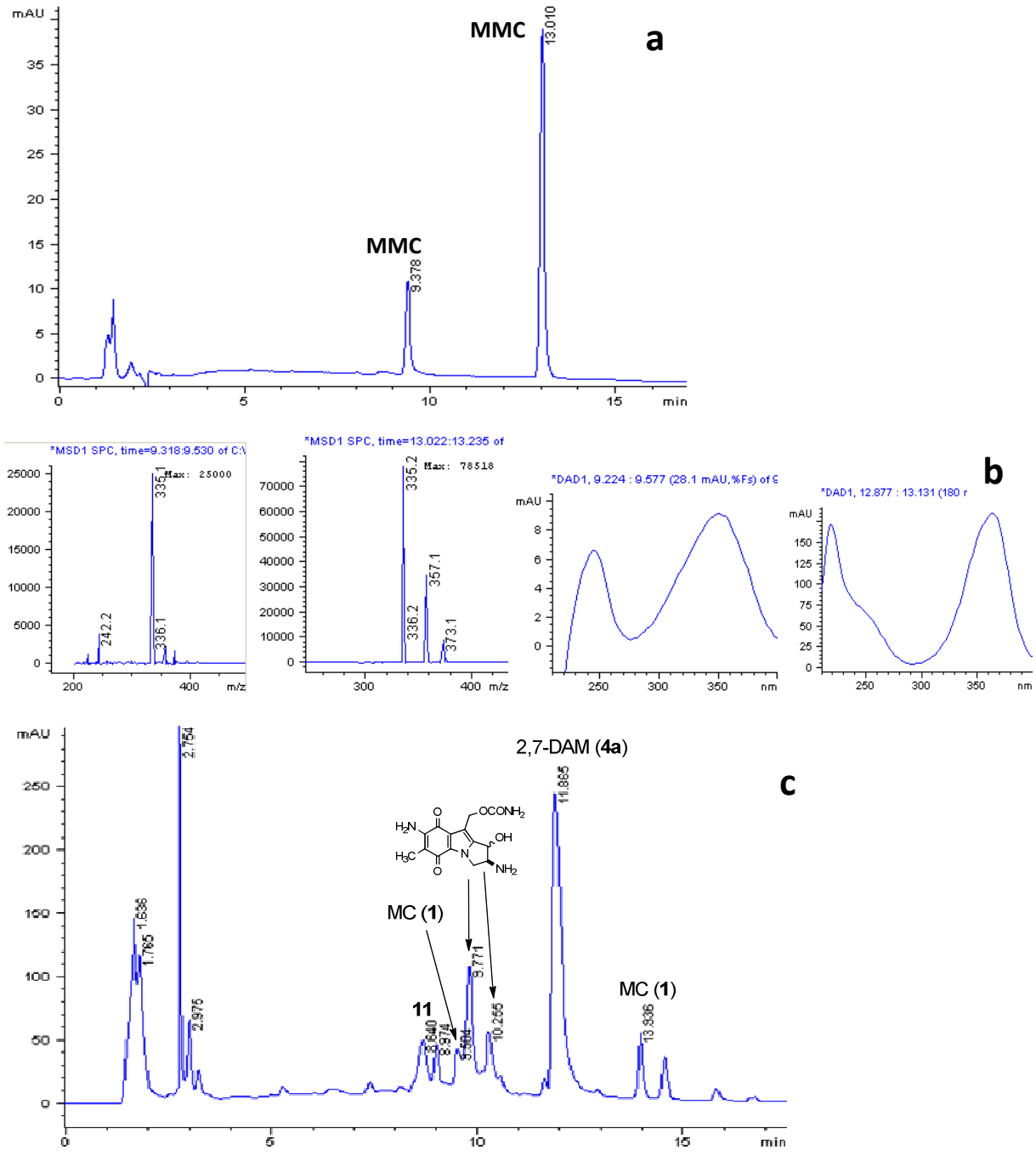
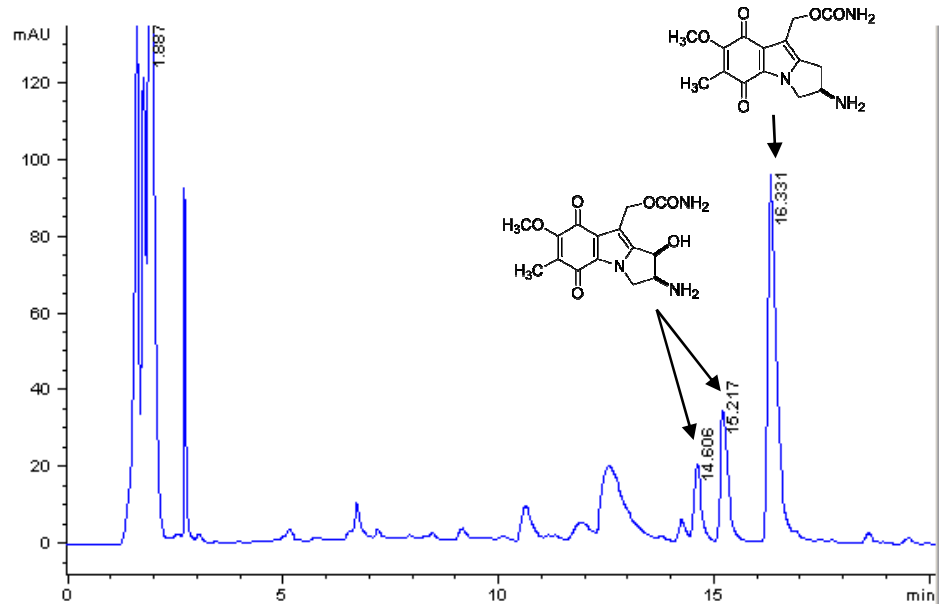
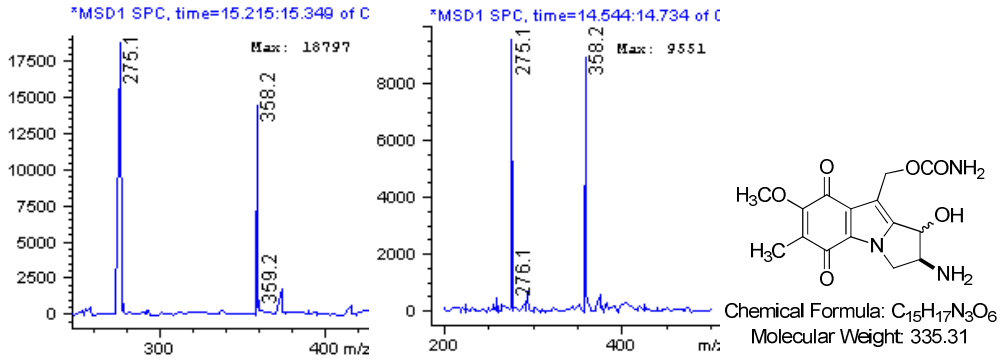


Figure S5. *MMA is converted to mitosenes by TrxR-NADPH*. Chromatogram (280 nm) from the LC/MS analysis of a reaction containing 300 nM TrxR, 20 µM MMA, 100 µM NADPH in TE buffer pH 7.0 after 24 h. Peaks were identified by comparing their retention times, UV, and MS spectra to those of mitosenes derived from MMA that were prepared by reduction of MMA with DTT as reported previously. (b) MS spectra of the major peaks observed in the chromatogram.



EI-MS (ES^+) *m/z*: 259 (M - $OCONH_2^-$), 342 (M + Na^+), 358 (M + K^+).



EI-MS (ES^+) *m/z*: 275 (M - $OCONH_2^-$), 358 (M + Na^+), 374 (M + K^+).

Figure S6. Determination of partition ratios for MMC and MMA from endpoint enzyme activity assays. The partition ratio was determined as described by Silverman (R. B. Silverman, Mechanism-Based Enzyme Inactivation and Enzymology, Vol. 1: Chemistry and Enzymology , CRC, Boca Raton, 1988). TrxR (100 nM) and NADPH (200 µM) were incubated with increasing concentrations of MMC. The enzyme activities were measured after 1 hour by the DTNB assay (A) and after 3 hours by using the insulin disulfide reduction assay (B). Similar experiments were performed using mitomycin A as inhibitor. Enzyme activity was determined by after 1 hour by the DTNB assay (C) and after 3 hours by using the insulin disulfide reduction assay (D). $[E_f]/[E_0]$ represents the relative enzyme activity for each concentration of inhibitor and $[I]/[E_0]$ represents the ratio concentration of inhibitor: initial concentration of enzyme.

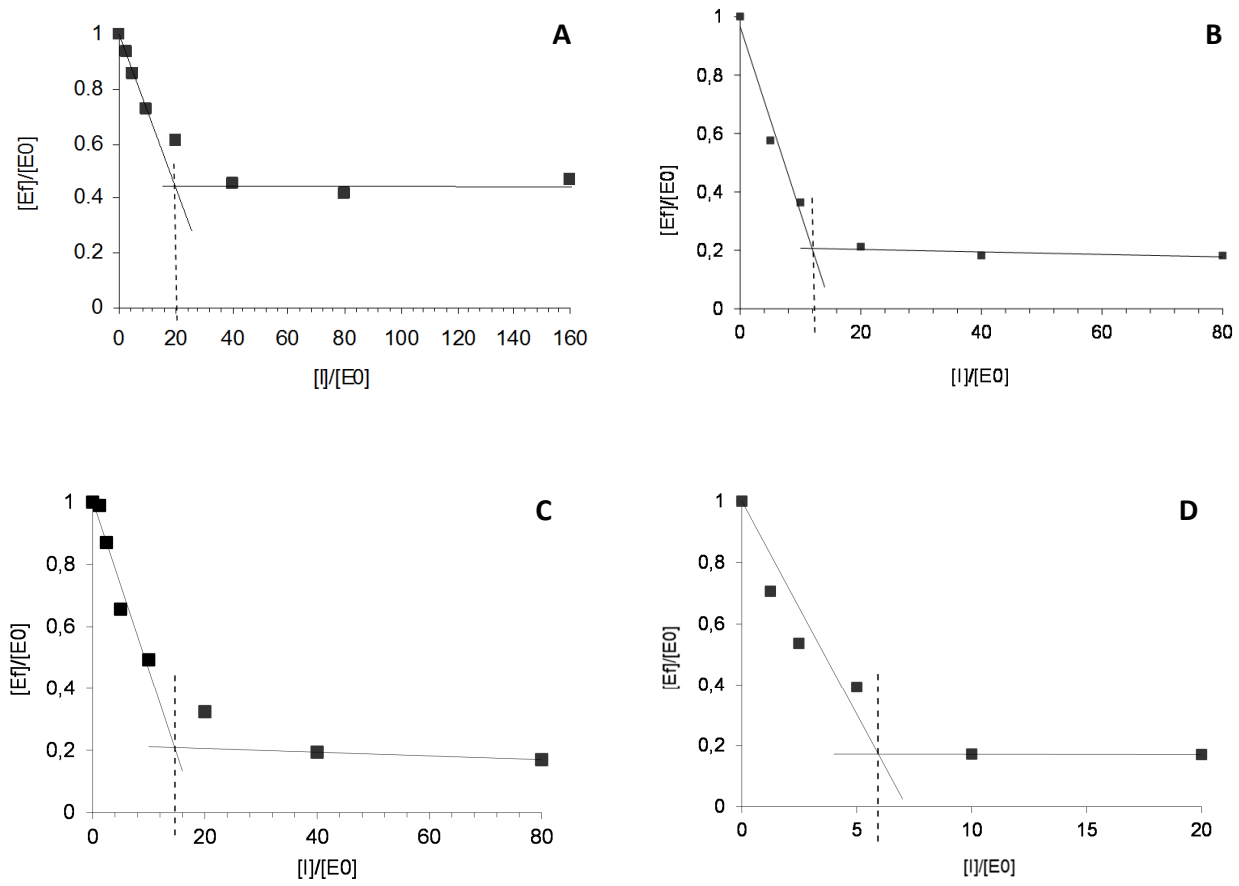


Figure S7. Effects of Mitomycin C on TrxR protein level and activity. Du145 cells were treated with various concentrations of mitomycin C for 24hrs. (A) Western blot analysis showing that TrxR expression decreases only slightly in cells treated with MMC.Aliquots of cell lysates were analyzed by Western blotting with anti-TrxR. (B) Relative levels of TrxR in cells treated with MMC calculated from the data shown in A. Relative band intensity refers to the band intensity of TrxR relative to that of actin (C) Effect of MMC on TrxR activity in the cells. TrxR activity assay was performed in 96-well plates by an endpoint insulin assay with 25 µg cell lysates.