

Supplementary Information

Selective Reduction of Methylsulfinyl-containing Compounds by mammalian MsrA Suggests a Strategy for Improved Drug Efficacy

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LEGENDS to Supplementary Figures

Figure S1. Expression and activity assays of Msr proteins. (A) Expressed and purified recombinant mouse MsrA (27.1 kDa), mouse MsrB2 (lacking the signal peptide) (15.8 kDa), and yeast fRMsr (20.8 kDa) proteins were analyzed by SDS-PAGE. (B) Specific activities of mouse MsrA for dabsylated Met-*S*-SO, mouse MsrB2 for dabsylated Met-*R*-SO, and yeast fRMsr for free Met-*R*-SO were determined using DTT used as a reductant. All measurements were repeated 3 times independently and shown with standard deviation.

Figure S2. Mesoridazine is reduced by mouse MsrA, but not by mouse MsrB2 or yeast fRMsr. Reduction of mesoridazine by mouse MsrA, mouse MsrB2 and yeast fRMsr in the presence of DTT was assayed using HPLC. (A) Mesoridazine reduction by mouse MsrA. (B) Mesoridazine reduction by mouse MsrB2. (C) Mesoridazine reduction by yeast fRMsr. (D) Mesoridazine reduction in the absence of enzymes

(control). In all panels, the original compound is marked with closed circles and the reduced form with stars.

Figure S3. Sulmazole is reduced by mouse MsrA, but not by mouse MsrB2 or yeast fRMs. Reduction of sulmazole by mouse MsrA, mouse MsrB2 and yeast fRMs was assayed using HPLC in a DTT-dependent reaction. (A) Sulmazole reduction by mouse MsrA. (B) HPLC analysis of sulmazole reduction by mouse MsrB2 with DTT. (C) Sulmazole reduction by yeast fRMs. (D) Sulmazole reduction in the absence of enzymes (control). In all panels, the original form is marked with closed circles and the reduced form (there is impurity in the original preparation) with stars.

Figure S4. Triclabendazole sulfoxide is reduced by mouse MsrA, but not by mouse MsrB2 or yeast fRMs. Reduction of triclabendazole sulfoxide by mouse MsrA, mouse MsrB2 and yeast fRMs was assayed using HPLC using TR/Trx/NADPH as a reduction system. (A) Triclabendazole sulfoxide reduction by mouse MsrA. (B) Triclabendazole sulfoxide reduction by mouse MsrB2. (C) Triclabendazole sulfoxide reduction by yeast fRMs. (D) Triclabendazole sulfoxide reduction in the absence of enzymes (control). In all panels, the original form is marked with closed circles and the reduced form with stars.

Figure S5. DMSO is reduced by mouse MsrA, but not by mouse MsrB2 or mouse MsrB1-Cys. Assays were performed in the presence of DTT and the products were analyzed by GC. (A) DMSO reduction by mouse MsrA. (B) DMSO reduction by mouse MsrB2. (C) DMSO reduction by mouse MsrB1-Cys. (D) DMSO reduction in the absence of enzymes (control). In all figures, the original form is marked with closed circles and the reduced form with stars.

Figure S6. Reduction of *R*- or *S*-sulforaphane-DTT by mouse MsrA and mouse MsrB2. Reduction of *R*- or *S*-sulforaphane-DTT by mouse MsrA and mouse MsrB2 was assayed using HPLC in the presence of DTT. (A) *R,S*-sulforaphane-DTT in the absence of enzymes (control). (B) *R*-sulforaphane-DTT reduction by mouse MsrB2. (C) *S*-sulforaphane-DTT reduction by mouse MsrA. In all panels, the original form is marked with closed circles and the reduced form with stars. All measurements were repeated 3 times

independently. (D) The reduction product of *R,S*-sulforaphane-DTT (in the absence of enzymes), *R*-sulforaphane-DTT in the presence of MsrB2, and *S*-sulforaphane-DTT in the presence of MsrA, were quantified at each time point (10, 20, 30, 40, 50, and 60 min).

Figure S7. Ion-spray mass spectrometry analysis of sulmazole reduction by Msrs. (A) Sulmazole reduction by mouse MsrA. Sulmazole is seen with m/z : 288.3 and its reduced form (sulmazole sulfide) with m/z : 272.3. (B) Sulmazole reduction by mouse MsrB2. (C) Sulmazole reduction by fRMsr.

Figure S8. Ion-spray mass spectrometry analysis of triclabendazole sulfoxide reduction by Msrs. (A) Triclabendazole sulfoxide reduction by mouse MsrA. Triclabendazole sulfoxide is seen with m/z : 375.1 and 377.1 and its reduced form (triclabendazole) with m/z : 359.1 and 361.1. (B) Triclabendazole sulfoxide reduction by mouse MsrB2. (C) Triclabendazole sulfoxide reduction by fRMsr.

Figure S9. Reduction of mesoridazine and oxidation of thioridazine. Mesoridazine was reduced to thioridazine with 80 μ g cytosolic fractions isolated from wild type, MsrA KO, and MsrB1 KO mouse liver. Reduction of sulmazole was assayed using HPLC in a DTT-dependent reaction. Thioridazine was oxidized to mesoridazine with 80 μ g microsomal fraction isolated from wild type mouse liver. Oxidation of thioridazine was assayed using HPLC. (A) HPLC analysis of mesoridazine reduction by the cytosolic fraction isolated from wild type mouse liver. (B) HPLC analysis of mesoridazine reduction by the cytosolic fraction isolated from MsrA KO mouse liver. (C) HPLC analysis of mesoridazine reduction by the cytosolic fraction isolated from MsrB1 KO mouse liver. (D) HPLC analysis of thioridazine oxidation by the microsomal fraction isolated from wild type mouse liver. The plot between 7 and 18 min is shown in the enlarged form in the inset with the peak of mesoridazine.

Figure S10. Reduction of sulmazole and mesoridazine by the cytosolic fraction and oxidation of thioridazine by the microsomes isolated from wild type, MsrA KO, and MsrB1 KO mouse liver. (A) Specific activity for the reduction of sulmazole by the cytosolic and microsomal fractions isolated from wild type, MsrA KO, and MsrB1 KO mouse liver. DTT was used as a reductant. (B) Specific activity for the reduction of mesoridazine by the cytosolic and microsomal fractions isolated from wild type, MsrA

KO, and MsrB1 KO mouse liver. DTT was used as a reductant. (C) Quantification of mesoridazine obtained by oxidation of thioridazine with microsomes isolated from wild type mouse liver. The reaction volume was 100 μ l and 40, 80, 120, and 160 μ g microsomes were used. (D) Specific activity for the oxidation of thioridazine to mesoridazine using microsomes isolated from wild type, MsrA KO, and MsrB1 KO mouse liver. All measurements were repeated 3 times independently and shown with standard deviation.

Figure S1

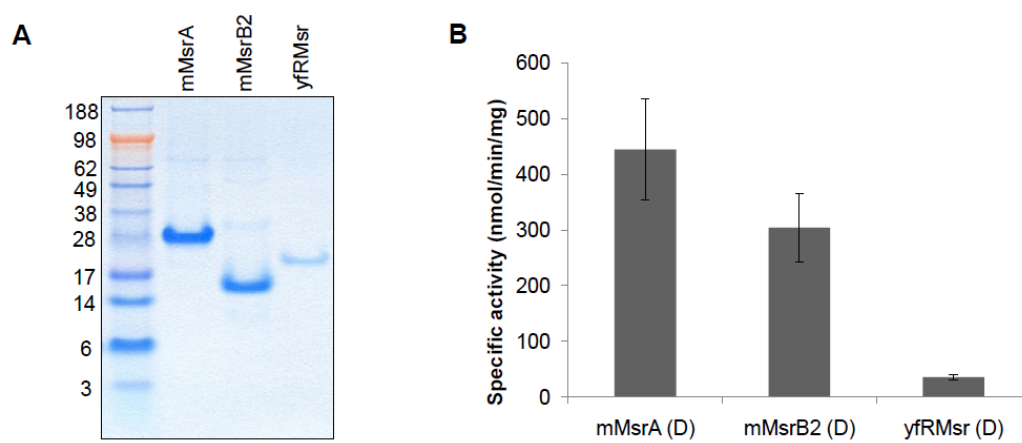


Figure S2

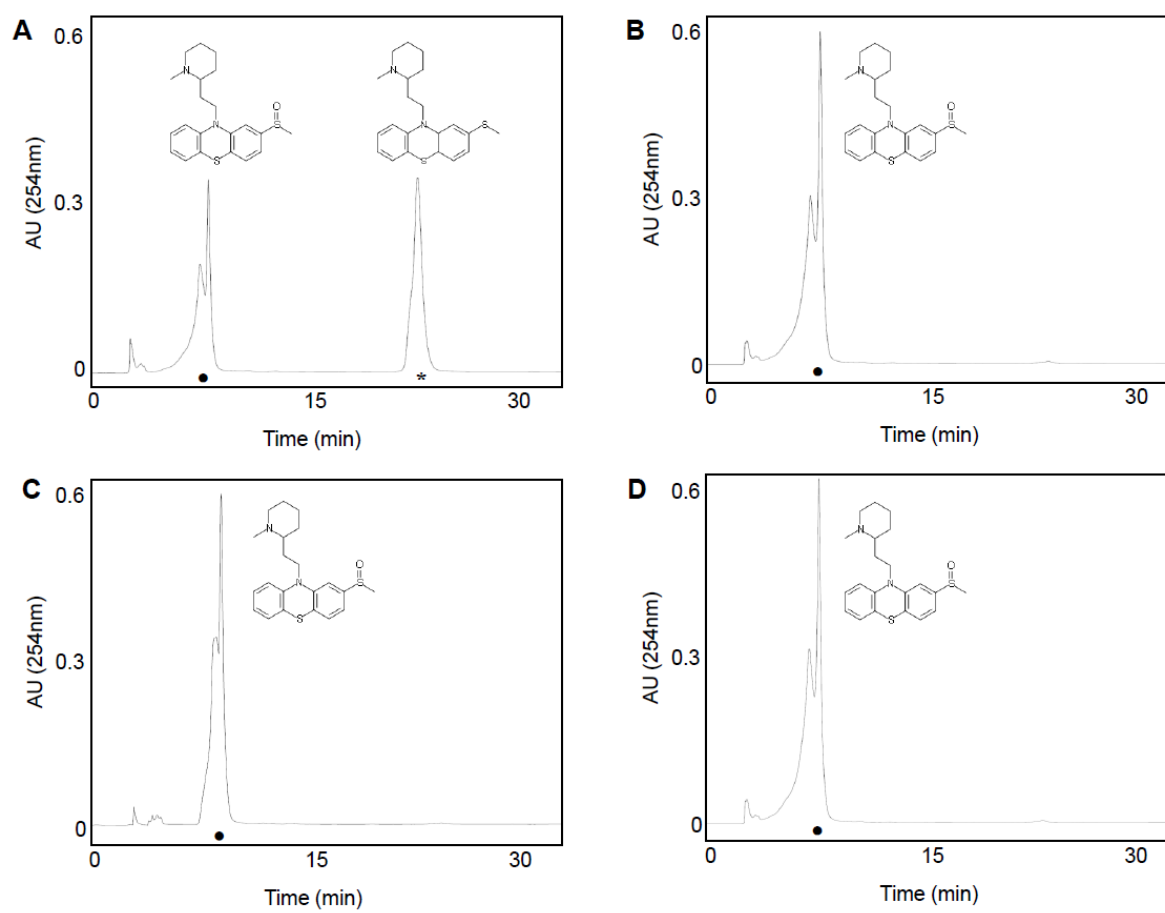


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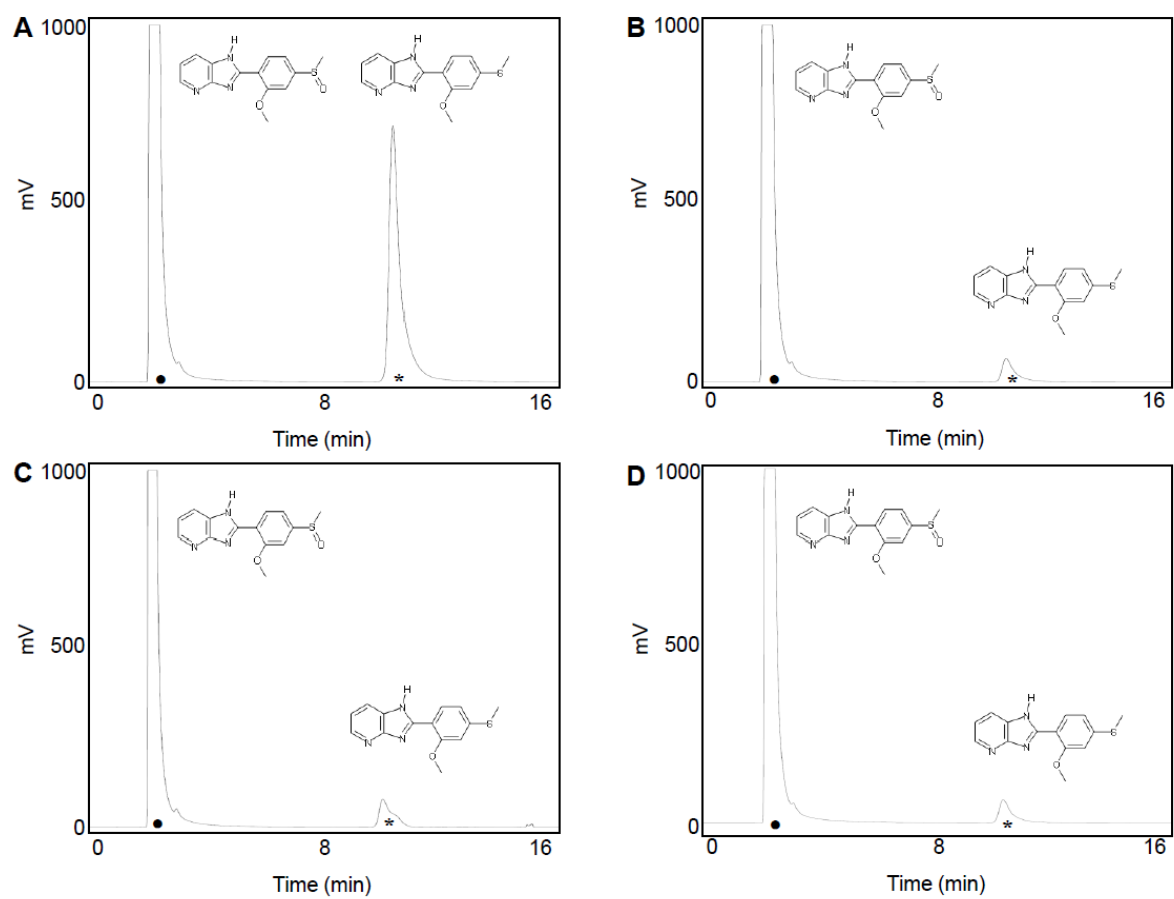


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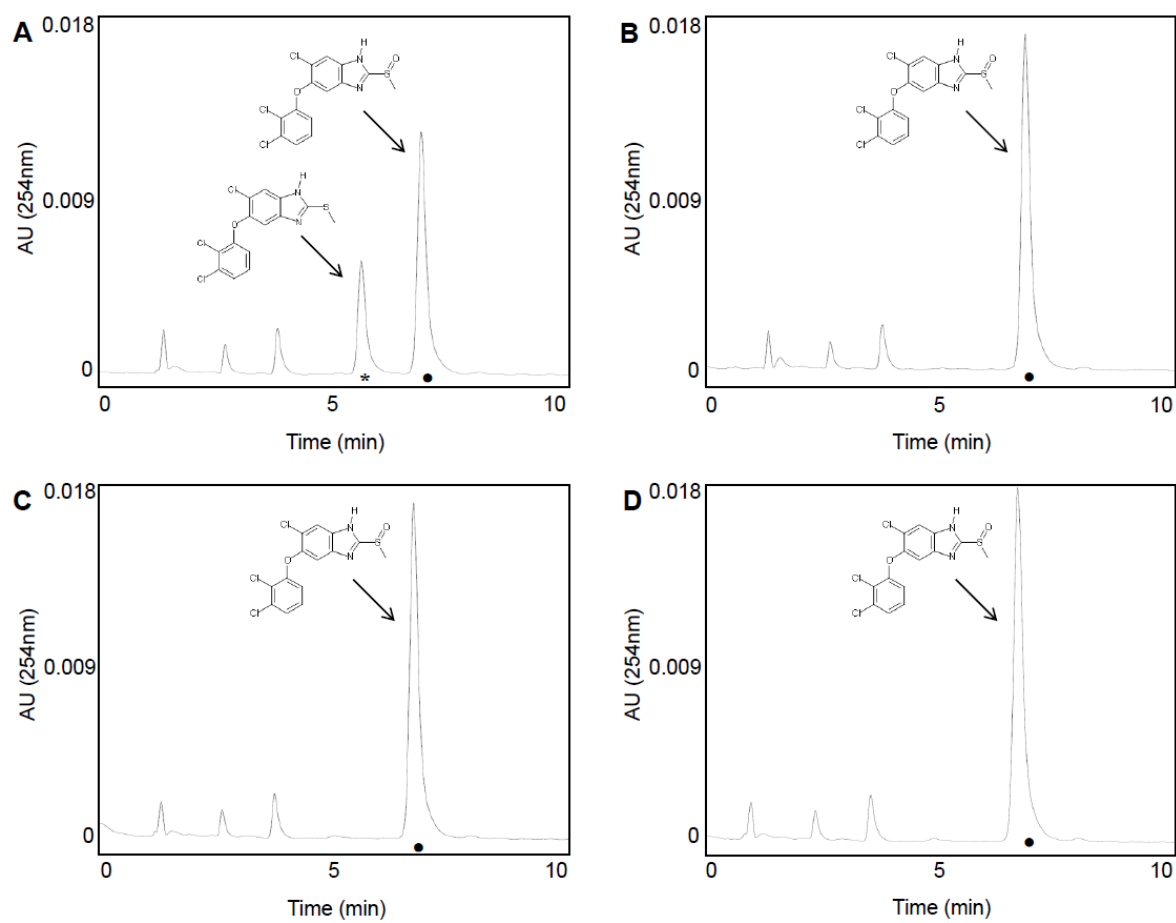


Figure S5

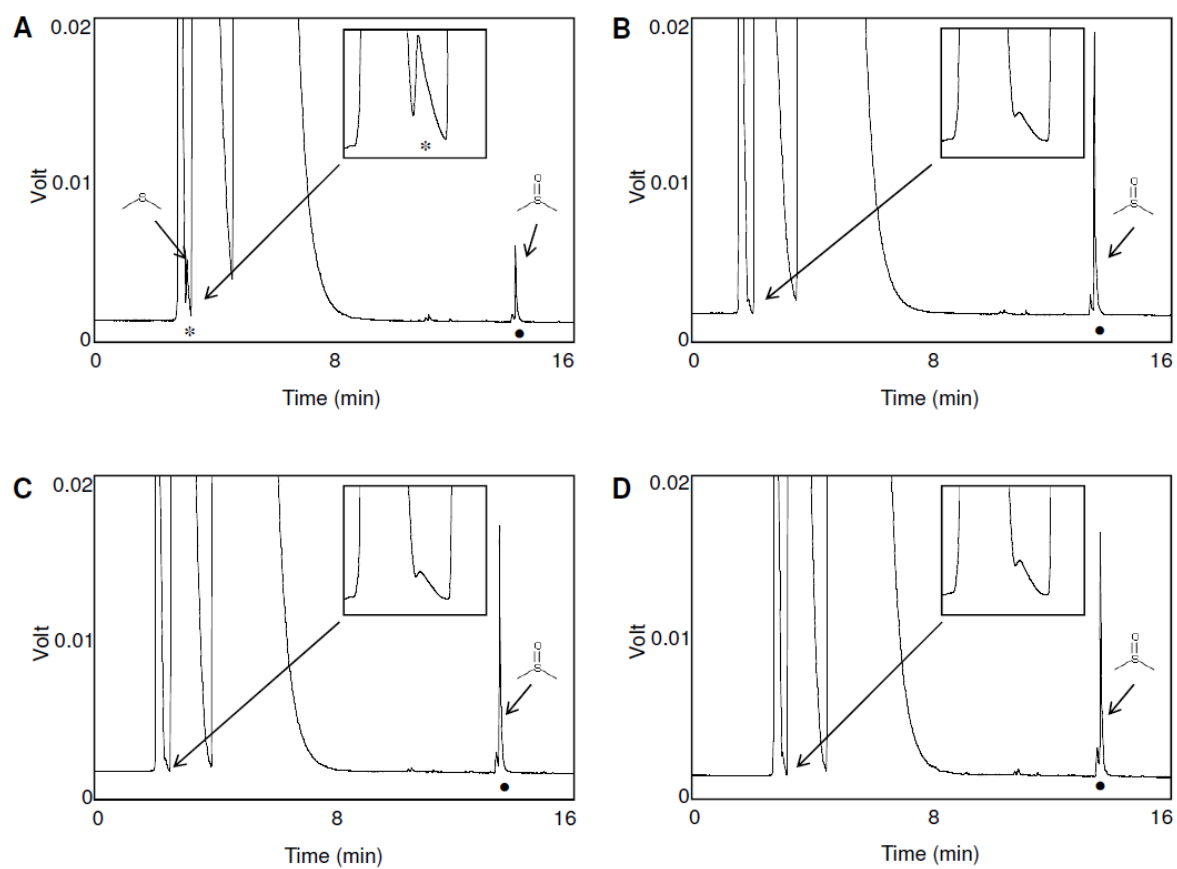


Figure S6

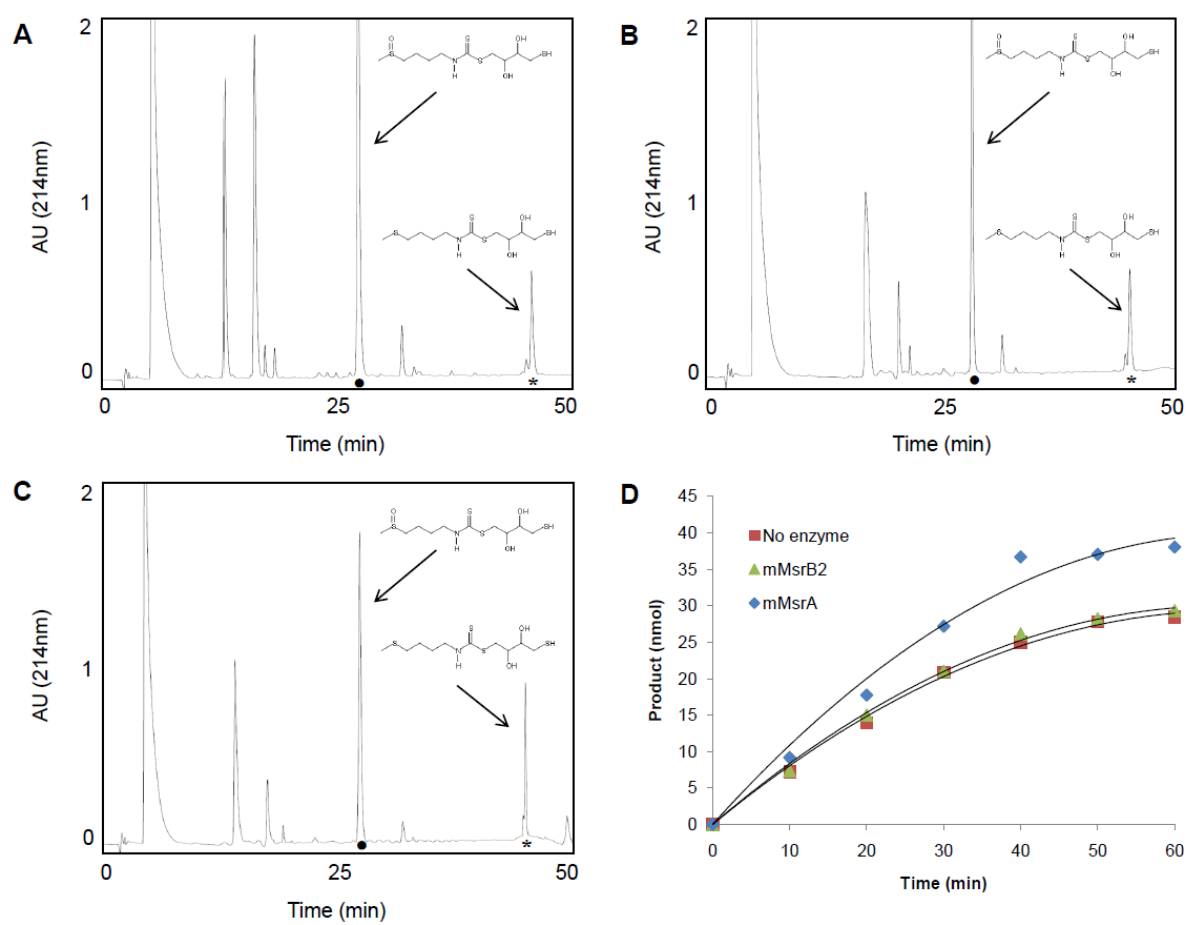


Figure S7

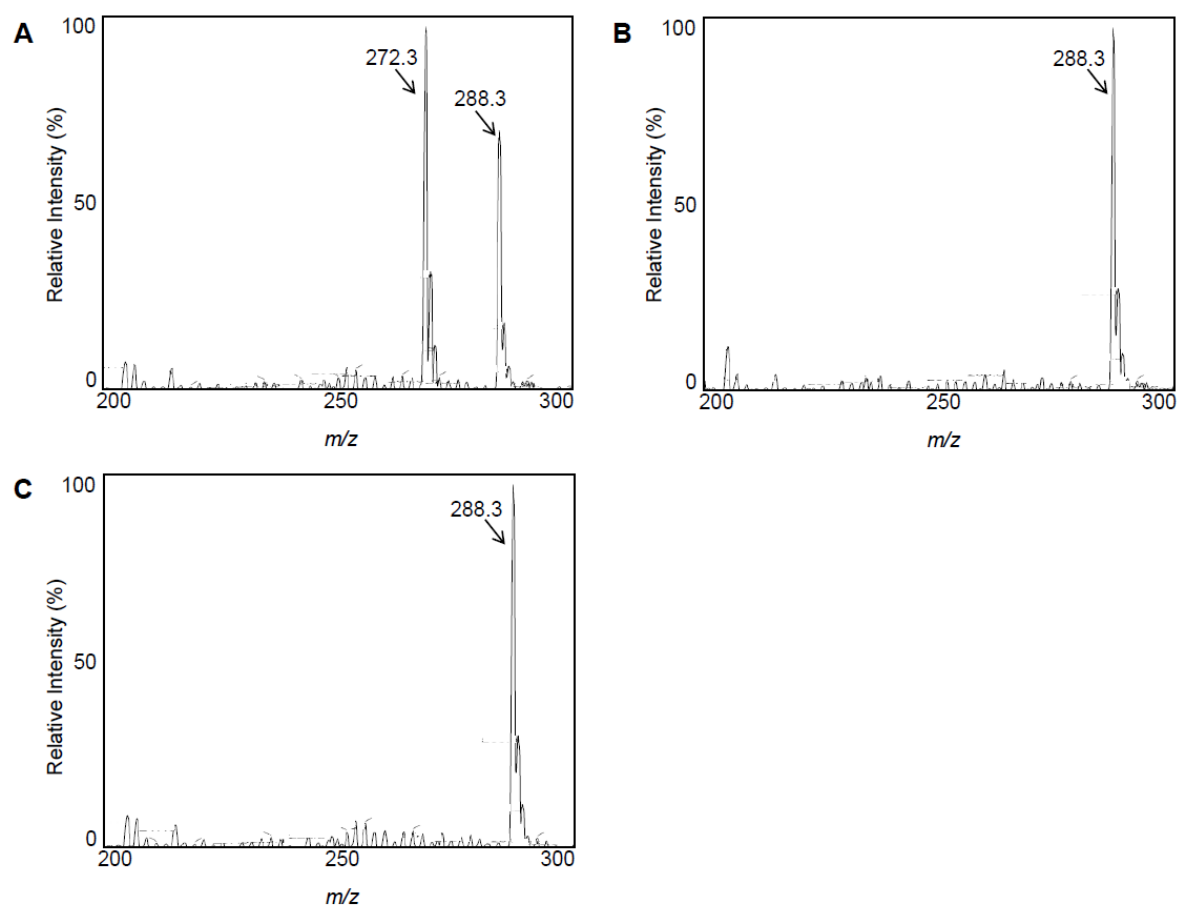


Figure S8

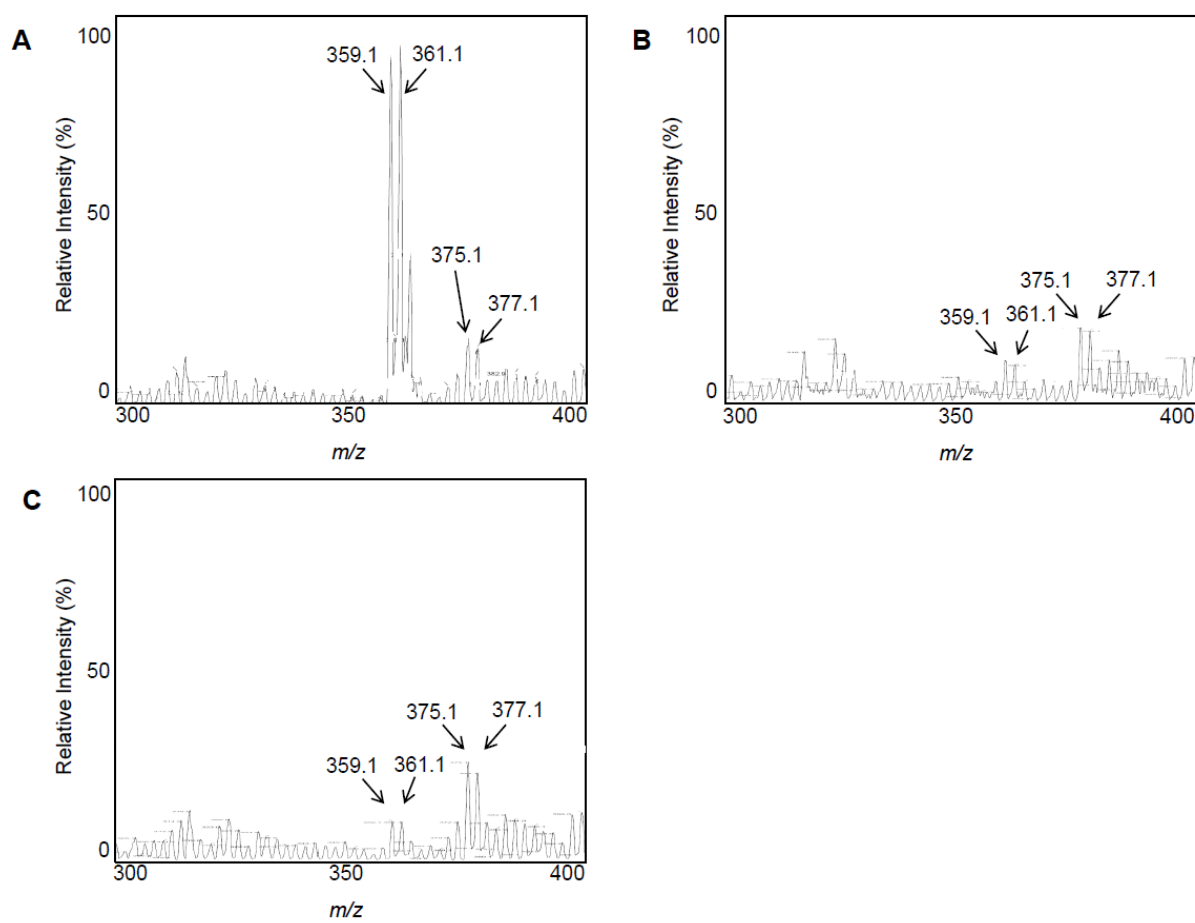


Figure S9

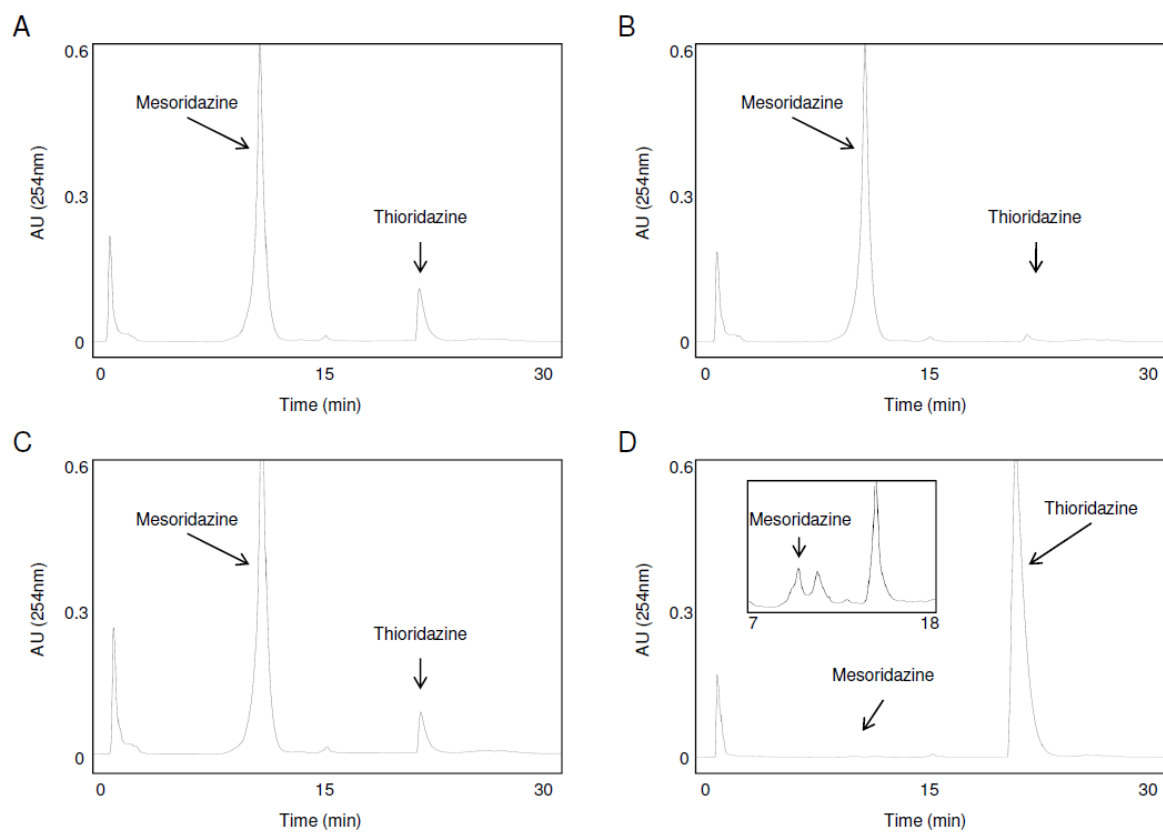


Figure S10

