Supplemental Data

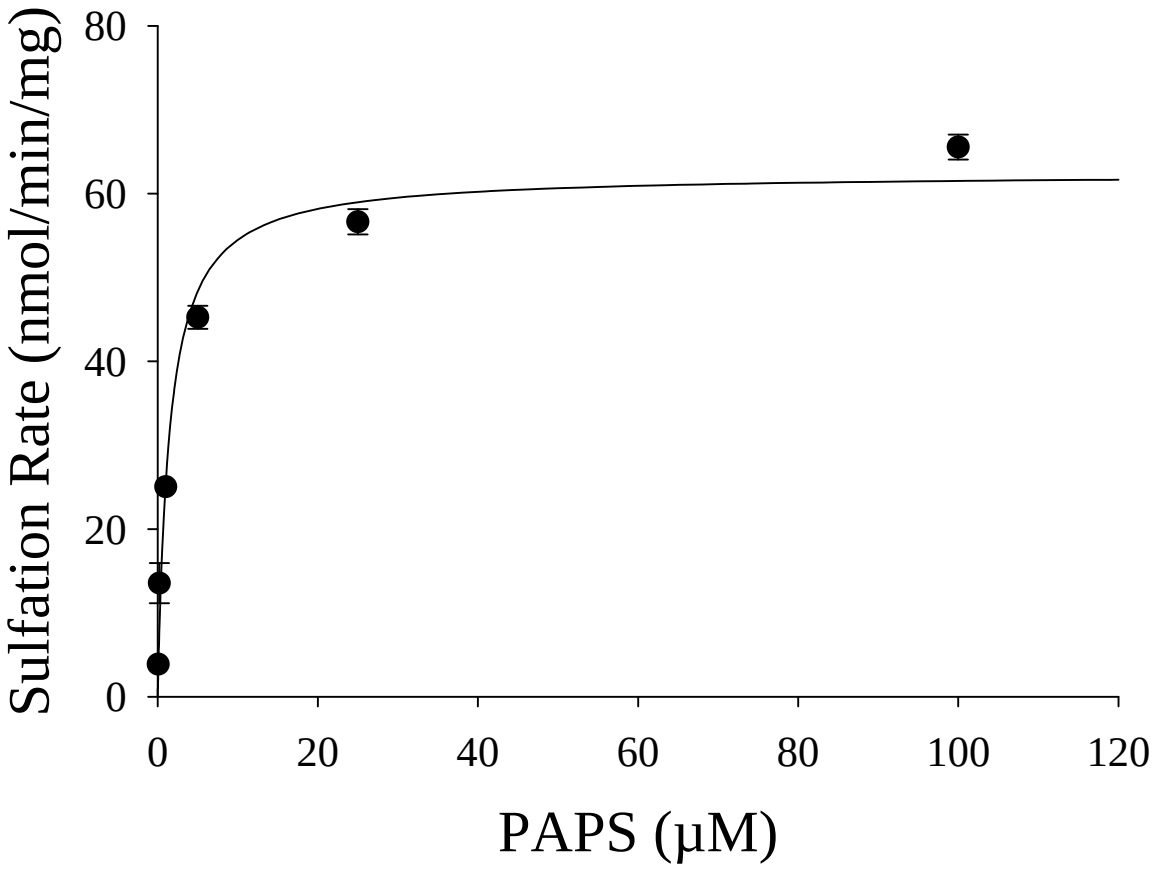
Drug Metabolism & Disposition

The Effects of Endoxifen and Other Major Metabolites of Tamoxifen on the Sulfation of Estradiol Catalyzed by Human Cytosolic Sulfotransferases hSULT1E1 and hSULT1A1*1

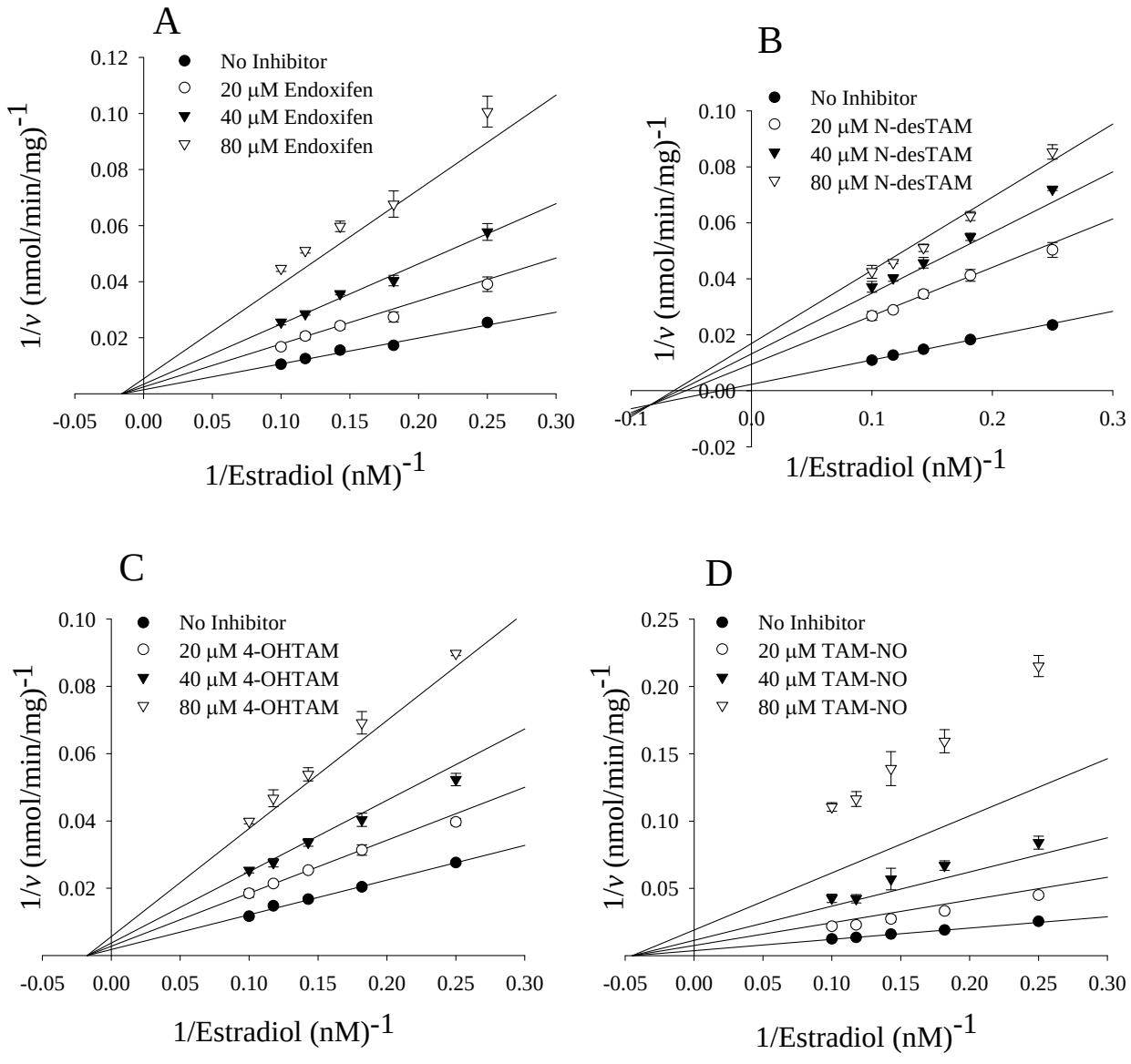
Edwin J. Squirewell and Michael W. Duffel*

Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

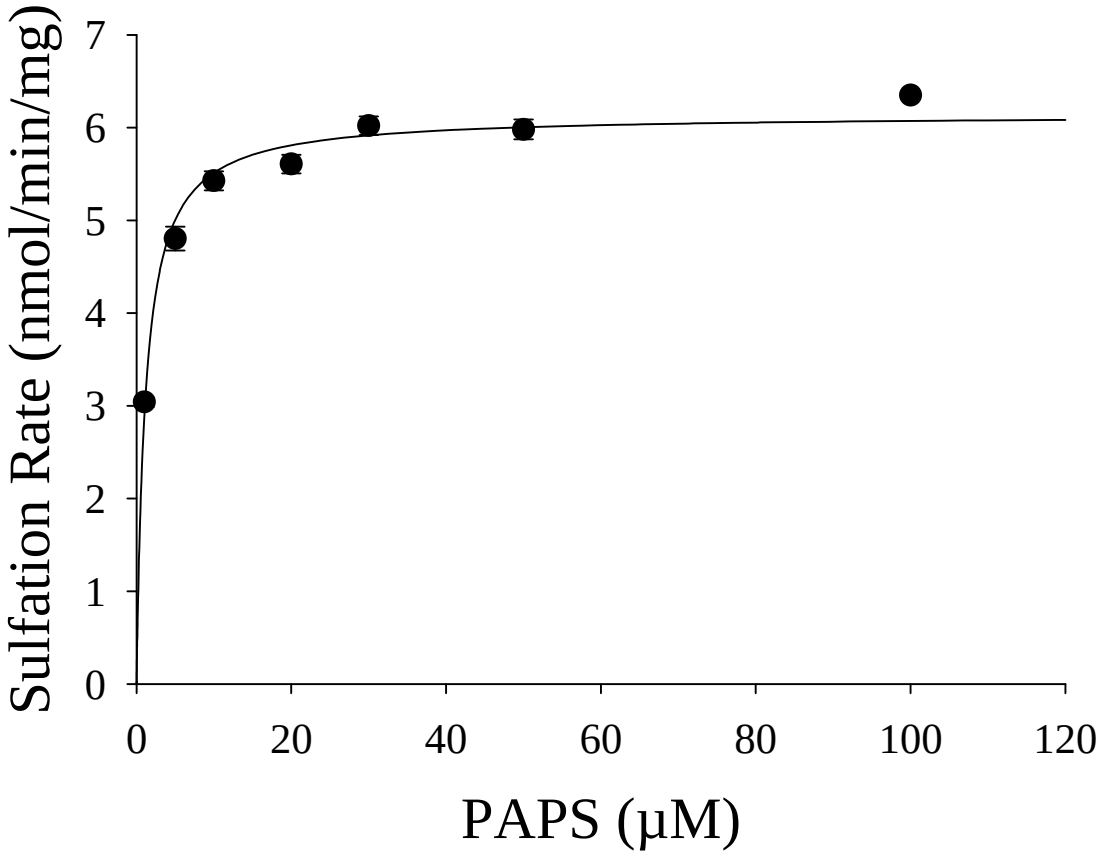
Supplemental Figure 1. Initial velocities of hSULT1E1-catalyzed sulfation of estradiol (50 μM) with varied concentrations of PAPS. The K_m and V_{max} for PAPS was 1.5 μM and 62 nmol/min/mg, respectively.



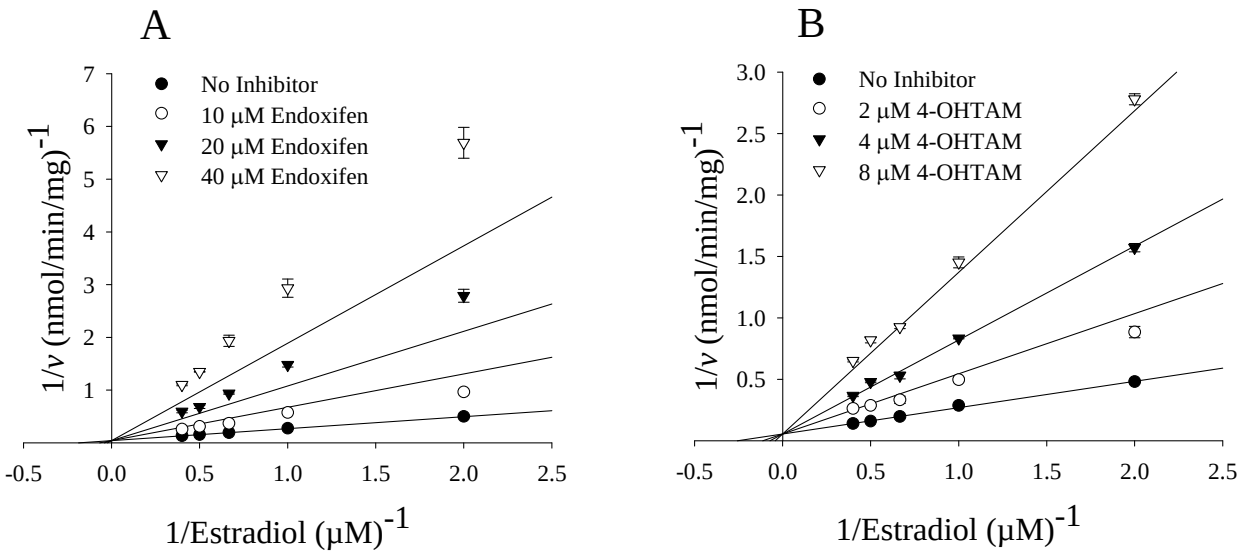
Supplemental Figure 2. K_i determinations for inhibitors of the hSULT1E1-catalyzed sulfation of estradiol. The inhibitors that were used in this study were (A) Endoxifen, (B) N-desTAM, (C) 4-OHTAM, and (D) TAM-NO. Each solid line represents a single concentration of inhibitor in the presence of various substrate concentrations, and data points are the means ± standard error from triplicate determinations. For A-C, the inhibition model (either non-competitive or mixed) that provided the best fit to the data is shown. While an attempted fit of the data to a non-competitive inhibition equation is shown for D, neither this nor other simple inhibition models adequately described inhibition at the higher concentration of TAM-NO.



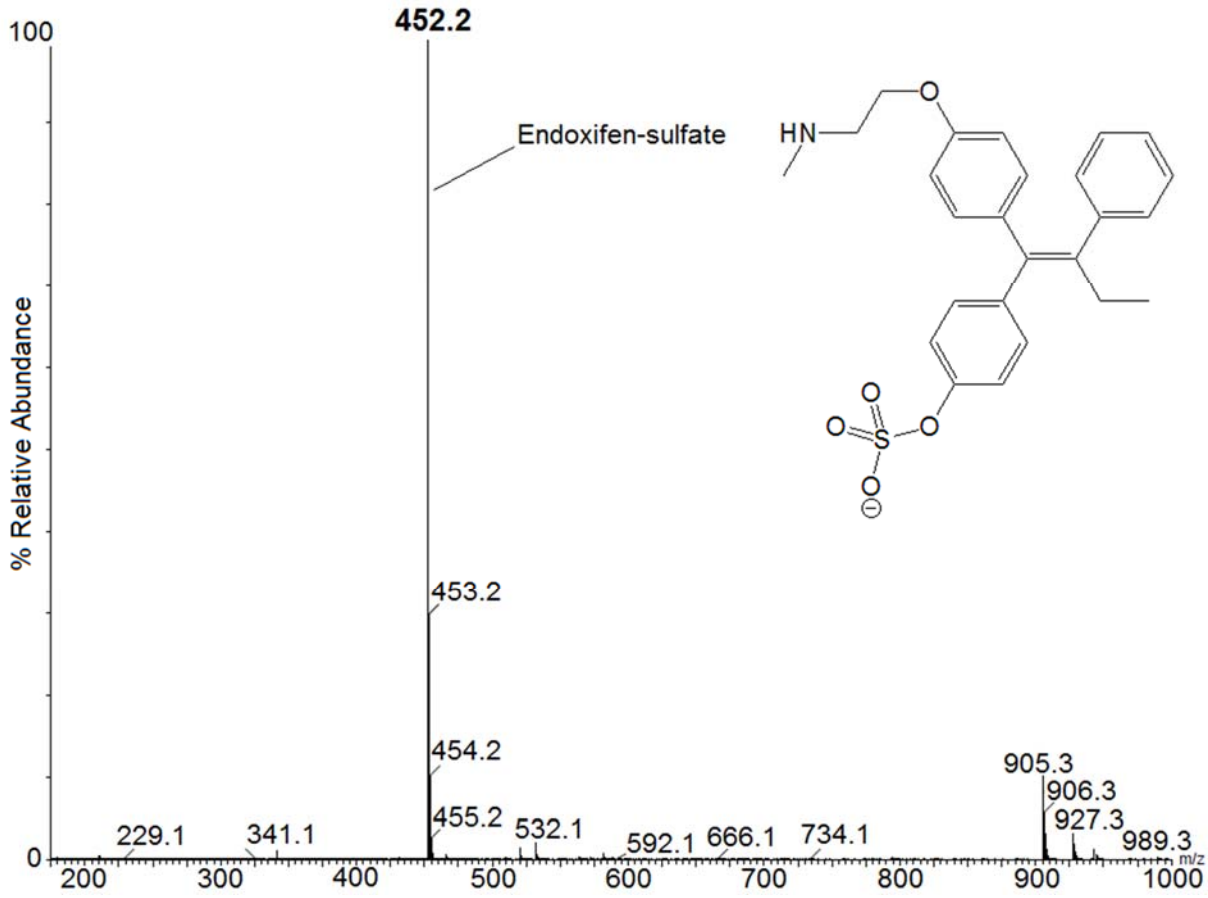
Supplemental Figure 3. Initial velocities of hSULT1A1*1-catalyzed sulfation of estradiol (5 μM) with varied concentrations of PAPS. The K_m and V_{max} for PAPS was 1.1 μM and 6.1 nmol/min/mg, respectively



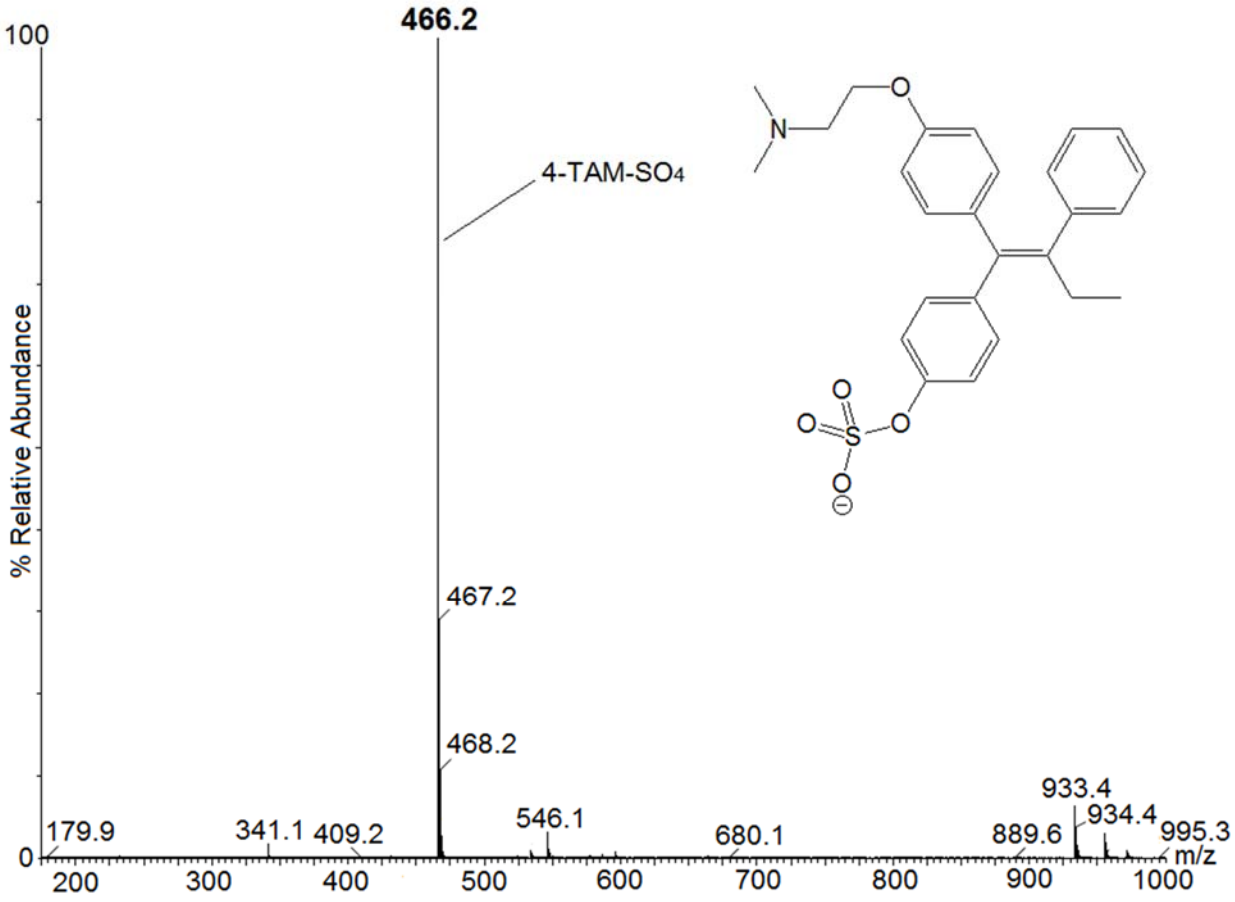
Supplemental Figure 4. K_i determinations for inhibitors of the hSULT1A1*1-catalyzed sulfation of estradiol. The inhibitors that were used in this study were (A) endoxifen and (B) 4-OHTAM. Each solid line represents a single concentration of inhibitor in the presence of various substrate concentrations, and data points are the means ± standard error from triplicate determinations. Data were fit using a competitive model for inhibition. As seen, the data for 4-OHTAM fit this model of inhibition well. However, the data for inhibition of the enzyme by endoxifen (A) did not fit a competitive inhibition model. Data for endoxifen as inhibitor also did not fit well to either non-competitive or mixed inhibition models under these assay conditions (not shown).



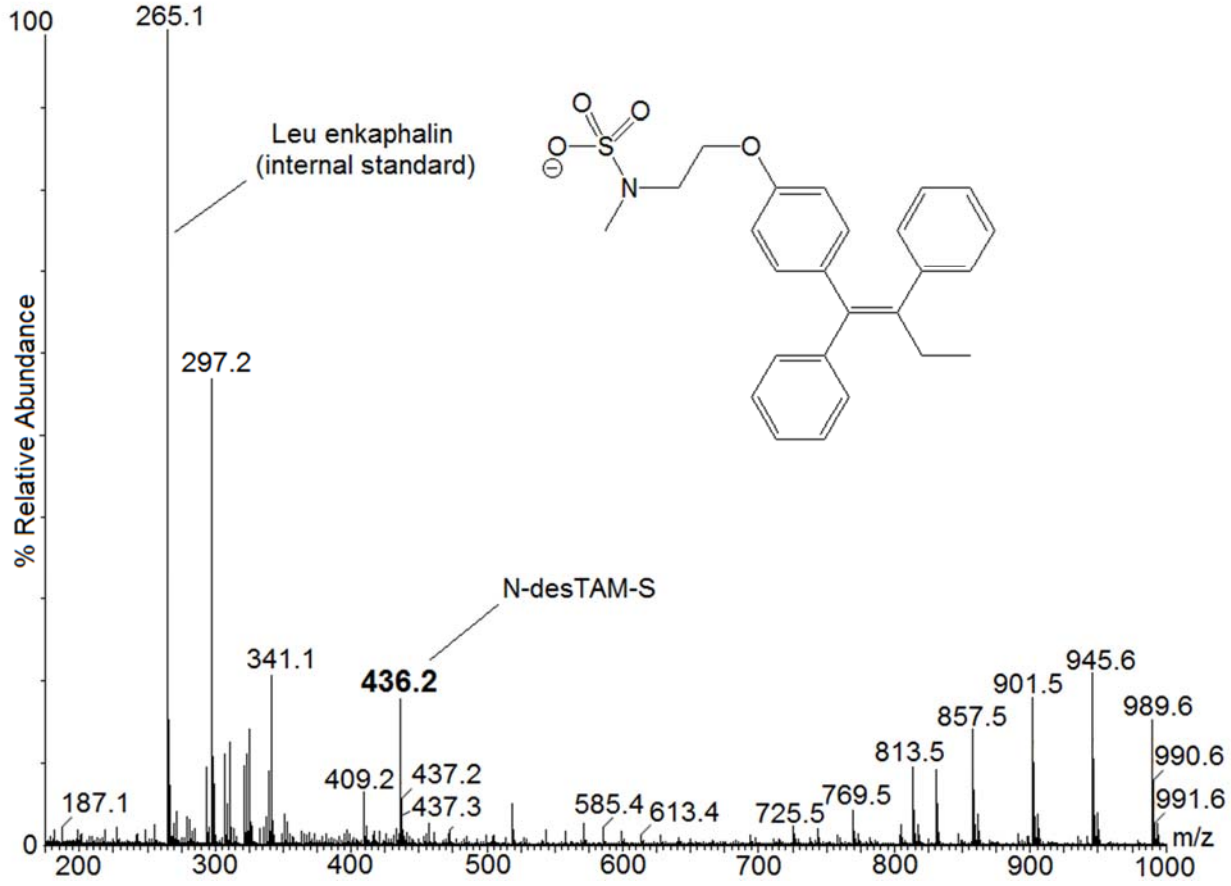
Supplemental Figure 5. LC-MS analysis of endoxifen sulfate formed in an enzymatic reaction catalyzed by hSULT1E1



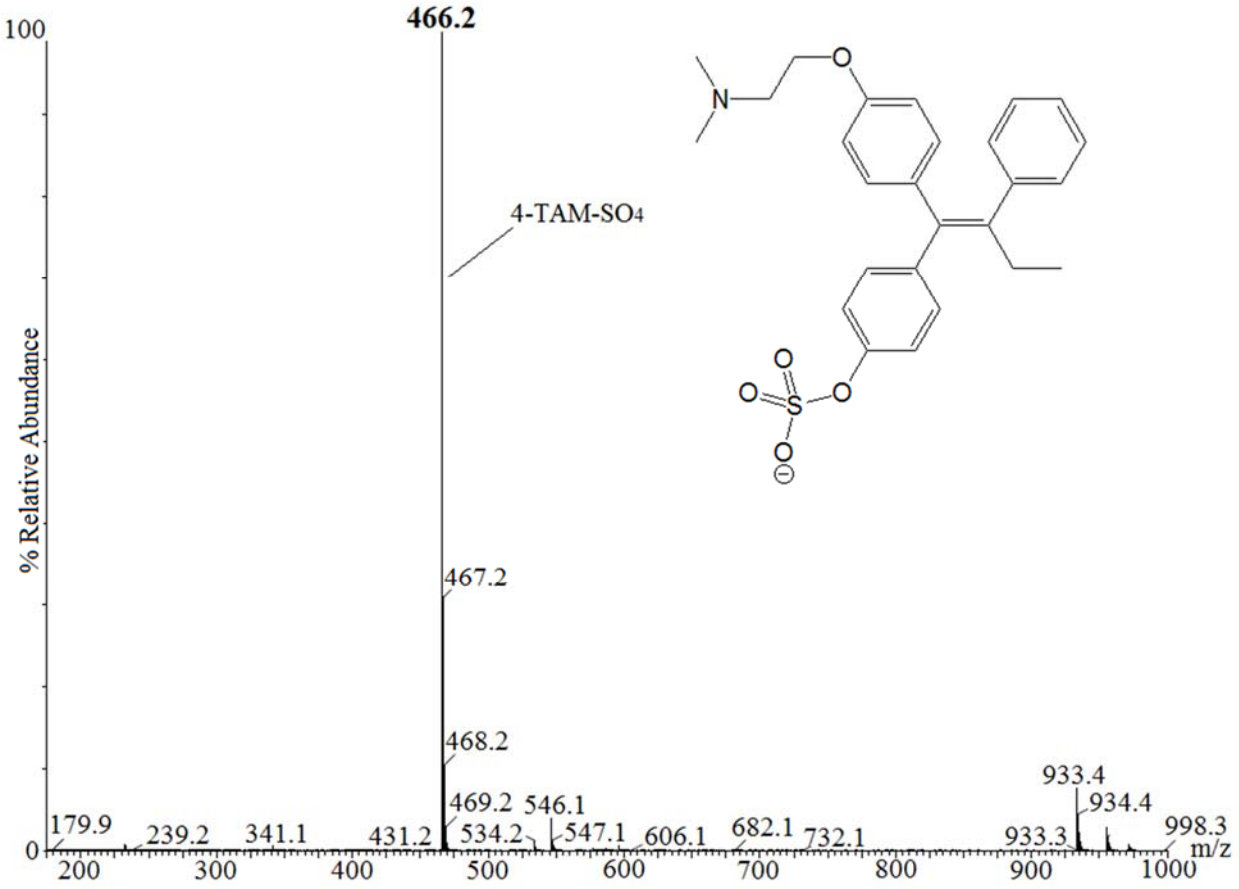
Supplemental Figure 6. LC-MS analysis of 4-TAM-SO_4 formed in an enzymatic reaction catalyzed by hSULT1E1



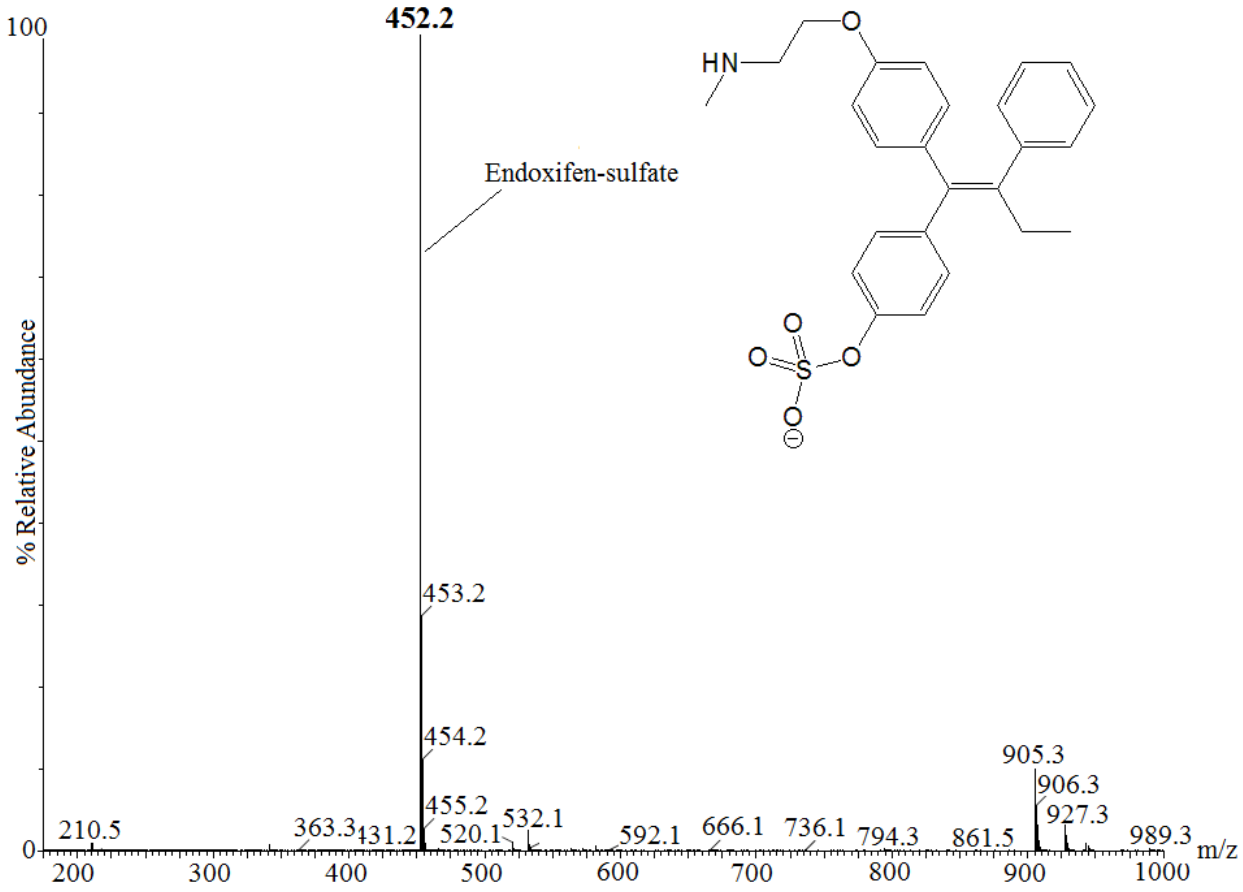
Supplemental Figure 7. LC-MS analysis of N-desTAM-S formed in an enzymatic reaction catalyzed by hSULT1E1.



Supplemental Figure 8. LC-MS analysis of 4-TAM-SO_4 formed in an enzymatic reaction catalyzed by hSULT1A1*1.



Supplemental Figure 9. LC-MS analysis of endoxifen-sulfate formed in an enzymatic reaction catalyzed by hSULT1A1*1.



Supplemental Figure 10. LC-MS analysis of N-desTAM formed in an enzymatic reaction catalyzed by hSULT1A1*1.