DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
DMD Fast Forward. Published on July 27, 2011 as doi:10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Investigation on the Enantioselectivity of the Sulfation of the Methylenedioxymethamphetamine (MDMA) Metabolites 3,4-Dihydroxymethamphetamine (DHMA) and 4-Hydroxy-3-Methoxymethamphetamine (HMMA) using the Substrate Depletion Approach

Andrea E. Schwaninger, Markus R. Meyer, and Hans H. Maurer

Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, D-66421 Homburg (Saar), Germany

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

Copyright 2011 by the American Society for Pharmacology and Experimental Therapeutics.

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Enantioselectivity of DHMA and HMMA Sulfation

Corresponding author:

Prof. Dr. Dr. h.c. Hans H. Maurer

Department of Experimental and Clinical Toxicology

Institute of Experimental and Clinical Pharmacology and Toxicology

Saarland University, Building 46, D-66421 Homburg (Saar), Germany

Tel.: +49-6841-1626050, Fax: +49-6841-1626051

Email: hans.maurer@uks.eu

Number of text pages: 12

Number of tables: 1

Number of figures: 2

Number of references: 29

Number of words in *Abstract*: 236

Number of words in *Introduction*: 370

Number of words in *Results and discussion*: 890

Abbreviations used are: MDMA, 3,4-methylenedioxymethamphetamine; CYP, cytochrome P450; DHMA, 3,4-dihydroxymethamphetamine; COMT, catechol-O-methyltransferase; HMMA, 4-hydroxy-3-methoxymethamphetamine; UGT, UDP-glucuronyltransferase; SULT, sulfotransferase; pHLC, pooled human liver cytosol; pholedrine; 4-hydroxymethamphetamine; DHBA, dihydroxybenzylamine; PAPS, adenosine 3'-phosphate 5'-phosphosulfate lithium salt hydrate; LC-HRMS, liquid chromatography-high resolution mass spectrometry; GC-NICI-MS, gas chromatography – negative ion chemical ionization – mass spectrometry

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Different pharmacokinetic properties are known for the two enantiomers of the entactogen 3,4-methylendioxy-methamphetamine (MDMA), most likely due to enantioselective metabolism. The aim of the present work was first, the investigation of the main sulfotransferases (SULT) isoenzymes involved in the sulfation of the main MDMA phase I metabolites 3,4-dihydroxymethamphetamine (DHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA) and second, the evaluation of a possible enantioselectivity of this phase II metabolic step. Therefore, racemic DHMA and HMMA were incubated with heterologously expressed SULTs and quantification of the sulfates by liquid chromatography-high resolution mass spectrometry was conducted. As separation of DHMA and HMMA sulfate could not be achieved by liquid chromatography, enantioselective kinetic parameters were determined using the substrate depletion approach with enantioselective quantification of substrate consumption by gas chromatography-negative ion chemical ionization mass spectrometry. SULT1A1 and SULT1A3 catalyzed sulfation of DHMA and SULT1A3 and SULT1E1 of HMMA. SULT1A1 and SULT1E1 revealed classic Michaelis-Menten kinetics whereas SULT1A3 kinetics showed deviation from the typical Michaelis-Menten kinetics resulting in a concentration-dependent self-inhibition. SULT1A3 showed the highest affinity and capacity of the SULT isoforms. Marked enantioselectivity could be observed for *S*-DHMA sulfation by SULT1A3 and in human liver cytosol, whereas no differences were observed for HMMA sulfation. Finally, comparison of Km and Vmax values calculated using achiral product formation and chiral substrate depletion showed good correlation within 2-fold of each other. In conclusion, preferences for S-enantiomers were observed for DHMA sulfation, but not for HMMA sulfation.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

The chiral compound *R,S*-3,4-Methylenedioxy-methamphetamine [*R,S*-MDMA, *R,S*-*N*-methyl-1-(3,4-methylenedioxyphenyl)propane-2-amine], also known as Ecstasy, is known as a very popular drug of abuse, but also associated with damage of serotonergic neurons (Kalant, 2001; Monks et al., 2004; de la Torre and Farre, 2004; Easton and Marsden, 2006). Metabolism of MDMA may play a role in this neurotoxicity (Miller et al., 1997; Bai et al., 1999; Mueller et al., 2009). As shown in Fig. 1, one major pathway of MDMA includes cytochrome P450 (CYP)-catalyzed *O*-demethylenation to 3,4-dihydroxymethamphetamine (DHMA), followed by *O*-methylation by catechol-*O*-methyltransferase (COMT) mainly to 4-hydroxy-3-methoxymethamphetamine (HMMA) and conjugation of DHMA and HMMA by sulfotransferases (SULTs) (Maurer, 1996; Maurer et al., 2000; de la Torre et al., 2004). In urine samples of recreational MDMA users, more than 90% of DHMA and HMMA are excreted as conjugates, with sulfates present in higher concentrations (Shima et al., 2008) (Schwaninger et al., Human MDMA and Phase I and Phase II Metabolite Urinary Excretion Kinetics Following Controlled MDMA Administration, in preparation). For the two enantiomers of MDMA, differences in their dose-response curves and in their *in vivo* kinetics were observed (Fallon et al., 1999; Kalant, 2001; Kraemer and Maurer, 2002; Pizarro et al., 2004; Peters et al., 2005). Enantioselective metabolism is the most likely explanation for the enantioselective pharmacokinetics of MDMA and was observed *in vitro* for CYP, COMT and UGT metabolism (Meyer et al., 2008; Schwaninger et al., 2009; Meyer and Maurer, 2009).

Enzyme kinetic data for metabolic steps represent important parameters in the understanding of (chiral) drug clearance. Conventional determination of enzyme kinetic parameters is based on assessing the product formation rate by quantification

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

of the formed metabolite. Therefore, availability of metabolite reference standards and suitable analytic techniques for chromatographic separation of the metabolites are necessary. An alternative was recently described for CYP enzymes by measuring the substrate depletion rate and was shown to be applicable for various typical CYP probe substrates (Obach and Reed-Hagen, 2002; Youdim and Dodia, 2010).

The aims of the present study was the identification of the human SULT isoforms involved in the formation of DHMA and HMMA sulfates and to elucidate enantioselective enzyme kinetic data in pooled human liver cytosol (pHLC) and heterologously expressed SULT isoforms with the substrate depletion approach.

Materials and Methods

Hydrochlorides of racemic MDMA, DHMA, and HMMA were obtained from Lipomed (Bad Saeckingen, Germany). 4-hydroxymethamphetamine (pholedrine), 3,4-dihydroxybenzylamine (DHBA), and adenosine 3'-phosphate 5'-phosphosulfate lithium salt hydrate (PAPS) were from Sigma Aldrich (Steinheim, Germany). DHMA 3-sulfate, DHMA 4-sulfate and HMMA sulfate were synthesized in the authors' laboratory as described previously (Schwaninger et al., 2011b). All other chemicals and reagents used were from Merck (Darmstadt, Germany) and of analytical grade. The following *E. coli*-derived recombinant human cytosolic SULT isoenzymes (rSULT, 20 µg) were from R&D Systems (Wiesbaden, Germany): SULT1A1, SULT1A3, SULT1B1, SULT1E1, SULT2A1. pHLC (20 mg protein/ml of 10 individual adult donors) was from BD Gentest (Heidelberg, Germany).

Product Formation Approach

Incubations were performed as described previously (Schwaninger et al., 2011b).

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

For initial screening experiments, 2.5 µM *R,S*-DHMA or *R,S*-HMMA and 10 µg/ml SULT1A1, SULT1A3, SULT1B1, SULT1E1, or SULT2A1 were incubated for 20 min. Kinetic constants of DHMA and HMMA sulfation were derived from incubations (n = 2) with the following substrate concentrations: 5, 10, 25, 50, 75, 100, 250 µM (SULT1A1) and 0.25, 0.5, 0.75, 1, 2.5, 5, 10 µM (SULT1A3) for DHMA and 0.2, 0.4, 0.6, 1, 1.5, 2.0, 2.5 (SULT1A3) and 5, 10, 25, 50, 75, 100, 250 (SULT1E1) for HMMA. Incubation times were 30 min (SULT1A1, SULT1E1) and 5 min (SULT1A3) and protein concentrations were 10 µg/ml for SULT1A1, 0.25 µg/ml for SULT1A3, and 1 µg/ml for SULT1E1 and were within the linear range of sulfate formation. Analysis was performed using liquid chromatography-high resolution mass spectrometry (LC-HRMS) as described previously (Schwaninger et al., 2011b). Enzyme kinetic constants were estimated by non-linear curve fitting using GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA). The Michaelis-Menten equation was used to calculate apparent V_{max} and K_m values.

Calculations of Net Clearance

The relative activity factor (RAF) approach was used to account for differences in expression between the two enzyme sources. The turnover rates for SULT1A1 (probe substrate nitrophenol, 0.25 µM), SULT1A3 (probe substrate dopamine, 1 µM), and SULT1E1 (probe substrate estradiol, 0.005 µM) were determined in rSULT and pHLC. Analysis was performed by LC-HRMS as described previously (Schwaninger et al., 2011b). The RAFs were calculated according to (Crespi and Miller, 1999; Venkatakrishnan et al., 2000; Grime and Riley, 2006). The enzyme velocities V_{enzyme} for the respective metabolic reactions were calculated at different substrate concentrations and were then multiplied with the corresponding RAF leading to the

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

contribution. From these contributions, the percentages of net clearance by a particular SULT at a certain substrate concentration were calculated.

Substrate Depletion Approach

Incubation mixtures (final volume, 275 µl) consisted of 100 mM phosphate buffer (pH 7.4), 5 mM $MgCl_2$, pHLC or SULT1A3, substrate, and PAPS. Aliquots of 50 µl were terminated by the addition of 50 µl of acetonitrile at different time points (0, 5, 10, 15, and 20 min). For determination of enantioselective kinetic constants the following substrate concentrations were used: 0.25, 0.5, 0.75, 1, 2.5, 5, and 10 µM for DHMA and 0.2, 0.4, 0.6, 1, 1.5, 2.0, and 2.5 µM for HMMA. PAPS concentration was 40 µM and protein concentrations were 0.5 µg/ml (SULT1A3) and 1 mg/ml (pHLC). After termination, 10 µl of IS solution (pholedrine and DHBA, 25 µM each) were added and the samples were worked-up and analyzed by gas chromatography-negative ion chemical ionization-mass spectrometry (GC-NICI-MS) as described previously (Schwaninger et al., 2011a). Analyte versus IS peak area ratios were determined and percentages of remaining substrate were calculated. The slope of the natural log of the percentage remaining over time represented the initial substrate depletion rates (k_{dep}) for each substrate concentration. If substrate decline demonstrated non-linearity on ln-percentage remaining versus time curves, only those initial time points wherein ln-linearity was observed, were used to determine k_{dep} (Obach and Reed-Hagen, 2002; Youdim and Dodia, 2010). K_m values were determined by plotting k_{dep} versus substrate concentration on a linear-log plot using Graph Pad Prism 5 and curve-fitting to equation 1 (Obach and Reed-Hagen, 2002),

$$K_{dep} = K_{dep}([S] = 0) \times (1 - \frac{[S]}{K_m + [S]}) \quad (1)$$

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

where [S] is the substrate concentration; K_m is the Michaelis-Menten constant and $k_{dep([S]=0)}$ is the theoretical maximum consumption rate constant at an infinitesimally low substrate concentration. Additionally, V_{max} and K_m values were estimated by simultaneous fitting to equation 2 (Youdim and Dodia, 2010).

$$-\frac{d[S]}{dt} = V = \frac{V_{max} \times [S]}{K_m + [S]} \quad (2)$$

V_{max} was normalized by dividing the calculated V_{max} (µM/min) by the actual incubation protein concentration. Stability of the substrates over the incubation period was determined at two concentrations (0.25 and 2.5 µM for DHMA and 0.2 and 1 µM for HMMA) as described above but without addition of PAPS. Instability would be indicated as a negative slope significantly different from zero ($p < 0.05$) by regression analysis plotting calculated concentrations versus time.

Correlation between Product Formation and Substrate Depletion

General applicability of the substrate depletion approach was checked by comparison of K_m and V_{max} values obtained for the two approaches. Therefore, in substrate depletion experiments, K_m and V_{max} were additionally calculated after summarizing the concentrations of both enantiomers to have comparable values to parameters obtained after achiral product formation measurements. For DHMA sulfation in the product formation approach, DHMA 3-sulfation and 4-sulfation were calculated as the sum of both sulfation reactions to give total DHMA sulfation.

Results and Discussion

Incubation conditions chosen were applicable to check the general involvement of the five major human SULT isoenzymes (Riches et al., 2009) in the sulfation of the MDMA metabolites DHMA and HMMA. Sulfation of DHMA was catalyzed by

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

SULT1A1 and SULT1A3 and sulfation of HMMA by SULT1A3 and SULT1E1 and their K_m, V_{max}, V_{max}/K_m values are listed in Table 1. The kinetic data for SULT1A1 and SULT1E1 followed classic Michaelis-Menten plots (Fig. 2A). In contrast, sulfation by SULT1A3 showed deviation from the typical Michaelis-Menten kinetics in which the substrate at higher concentrations had a marked concentration-dependent self-inhibition. To obtain comparable kinetic values, they were calculated by the classic Michaelis-Menten equation within a concentration range in which substrate inhibition was not of relevance (0.25-10 µM for DHMA; 0.2-2.5 µM for HMMA). DHMA sulfation could be observed in position 3 and position 4 by SULT1A3, with higher turnover rates for position 3. For SULT1A1, only sulfation in position 3 could be observed. However it should be considered that sulfation by SULT1A1 was rather low which might result in formation of DHMA 4-sulfate below the detection limit of the used LC-HRMS method.

SULT1A3 turned out to have the highest affinity and the highest capacity for DHMA and HMMA sulfation with V_{max}/K_m values about 5000 or 3000 fold higher than those for SULT1A1 or SULT1E1, respectively (Table 1). For assessment of contribution of SULT1A1, SULT1A3, and SULT1E1 in pHLC, the RAF approach was used (Crespi and Miller, 1999; Venkatakrishnan et al., 2000; Grime and Riley, 2006) to account for differences in protein expression. Nitrophenol, dopamine and estradiol were used as probe substrates to calculate RAFs and were accepted to be selective for the described SULT isoforms (Zhang et al., 1998; Dajani et al., 1999; Wang and James, 2005; Riches et al., 2009). However some drawbacks such as the involvement of other isoforms at higher substrate concentrations should be considered. Concentrations of the probe substrates were therefore chosen to be selective for the single SULT isoforms. Although SULT1A3 was claimed to be only scarcely

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

expressed in human liver (Riches et al., 2009), DHMA and HMMA were predominantly catalyzed by SULT1A3 (calculated net clearance > 90%) at low substrate concentration. Increasing substrate concentration to 10 µM resulted in decreasing contribution of SULT1A3 to about 70% for DHMA whereas contribution for HMMA sulfation remained more than 90%.

The most likely explanation for the observed different pharmacokinetic properties of *R*- and *S*-MDMA is an enantioselective metabolism. Enantioselectivity was observed for CYP2C19 (Meyer et al., 2008), COMT (Meyer and Maurer, 2009), UGT1A9 and UGT2B17 (Schwaninger et al., 2009), always with preferences for the *S*-enantiomers. Although sulfation of MDMA metabolites was already studied and was found to be the major phase II metabolic step (Schwaninger et al., 2011b), no data concerning enantioselectivity are available. As stereoselective separation of DHMA and HMMA sulfates could not be achieved, a substrate depletion approach was used, where the depletion of DHMA and HMMA was measured enantioselectively by GC-MS (Schwaninger et al., 2011a). DHMA and HMMA were stable over the incubation time of 20 min. The K_m, V_{max}, V_{max}/K_m values are listed in Table 1. As shown in the sigmoidal plots in Fig. 2B, marked enantioselectivity (defined as $V_{max}/K_{m\ (S\text{-enantiomer})}/V_{max}/K_{m\ (R\text{-enantiomer})} > 1.5$) according to Meyer et al. (Meyer et al., 2008) was observed for DHMA sulfation by SULT1A3 toward *S*-DHMA. No preferences could be observed for HMMA sulfation by SULT1A3. One reason for this difference in enantioselectivity might be the position for sulfation. DHMA was mainly sulfated in position 3, whereas HMMA could only be sulfated in position 4. Comparable *S/R* ratios as with SULT1A3 were calculated in pHLC, indicating that *in vivo* enantioselectivity with preferences for the *S*-enantiomer could be expected for DHMA 3-sulfate, but not for HMMA sulfate. Chiral analysis of urine samples of MDMA users

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

analyzed after conjugate cleavage revealed preferences for *S*-DHMA, but no differences between *R*- and *S*-HMMA (Pizarro et al., 2004). That is in line with the *in vitro* observation that sulfation of HMMA as the major phase II metabolic step showed no enantioselectivity.

The general applicability for determination of enantioselective sulfation reactions was checked by comparing kinetic parameters calculated using the product formation and the substrate depletion approach. In the product formation approach, K_m and V_{max} values for DHMA 3-sulfation and 4-sulfation were calculated after summarizing the concentrations of DHMA 3- and DHMA 4-sulfate to give total DHMA sulfation. For the substrate depletion experiments, K_m and V_{max} values were calculated after summarizing the concentrations of both enantiomers to have comparable values to parameters obtained after achiral product formation measurements. As shown in Fig. 2C, good correlation, with K_m and V_{max} values within 2-fold of each other, was observed between product formation and substrate depletion experiments using both, recombinant SULT and pHLC. However, the substrate depletion approach possesses some practical limitations (Obach and Reed-Hagen, 2002), such as compounds with low turnover rates and the lack of differentiation between formation of several metabolites. Therefore, it could not be used for SULT1A1 and SULT1E1, as no substantial amount of the initial substrate concentration was consumed during the incubation period. Furthermore, differentiation between sulfation to DHMA 3-sulfate and DHMA 4-sulfate was not possible by monitoring substrate loss. However, product formation experiments showed that sulfation mainly took place in position 3 and DHMA 4-sulfate was only a minor metabolite which might be neglected.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgements

We thank Carsten Schröder, Gabriele Ulrich, Armin Weber, and Carina Wink for assistance and helpful discussions.

Authorship Contributions

Participated in research design: Schwaninger, Meyer, Maurer

Conducted experiments: Schwaninger

Performed data analysis: Schwaninger

Wrote or contributed to the writing of the manuscript: Schwaninger, Meyer, Maurer

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

References

Bai F, Lau SS and Monks TJ (1999) Glutathione and N-acetylcysteine conjugates of alpha-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxyamphetamine-mediated neurotoxicity. *Chem Res Toxicol* **12**:1150-1157.

Crespi CL and Miller VP (1999) The use of heterologously expressed drug metabolizing enzymes-state of the art and prospects for the future. *Pharmacol Ther* **84**:121-131.

Dajani R, Cleasby A, Neu M, Wonacott AJ, Jhoti H, Hood AM, Modi S, Hersey A, Taskinen J, Cooke RM, Manchee GR and Coughtrie MW (1999) X-ray crystal structure of human dopamine sulfotransferase, SULT1A3. Molecular modeling and quantitative structure-activity relationship analysis demonstrate a molecular basis for sulfotransferase substrate specificity. *J Biol Chem* **274**:37862-37868.

de la Torre R and Farre M (2004) Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. *Trends Pharmacol Sci* **25**:505-508.

de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M, Segura J and Cami J (2004) Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition [review]. *Ther Drug Monit* **26**:137-144.

Easton N and Marsden CA (2006) Ecstasy: are animal data consistent between species and can they translate to humans? [review]. *J Psychopharmacol* **20**:194-210.

Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA and Hutt AJ (1999) Stereospecific analysis and enantiomeric disposition of 3, 4-methylenedioxymethamphetamine (Ecstasy) in humans. *Clin Chem* **45**:1058-1069.

Grime K and Riley RJ (2006) The impact of in vitro binding on in vitro-in vivo extrapolations, projections of metabolic clearance and clinical drug-drug interactions. *Curr Drug Metab* **7**:251-264.

Kalant H (2001) The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs [review]. *Can Med Assoc J* **165**:917-928.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Kraemer T and Maurer HH (2002) Toxicokinetics of amphetamines: Metabolism and toxicokinetic data of designer drugs, of amphetamine, methamphetamine and their N-alkyl derivatives [review]. *Ther Drug Monit* **24**:277-289.

Maurer HH (1996) On the metabolism and the toxicological analysis of methylenedioxyphenylalkylamine designer drugs by gas chromatography-mass spectrometry. *Ther Drug Monit* **18**:465-470.

Maurer HH, Bickeboeller-Friedrich J, Kraemer T and Peters FT (2000) Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ("Ecstasy"). *Toxicol Lett* **112**:133-142.

Meyer MR and Maurer HH (2009) Enantioselectivity in the Methylation of the Catecholic Phase-I Metabolites of Methylenedioxy Designer Drugs and their Capability to Inhibit COMT Catalyzed Dopamine 3-Methylation. *Chem Res Toxicol* **22**:1205-1211.

Meyer MR, Peters FT and Maurer HH (2008) The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxy-methamphetamine and its enantiomers. *Drug Metab Dispos* **36**:2345-2354.

Miller RT, Lau SS and Monks TJ (1997) 2,5-Bis-(glutathion-S-yl)-alpha-methyldopamine, a putative metabolite of (+/-)-3,4-methylenedioxyamphetamine, decreases brain serotonin concentrations. *Eur J Pharmacol* **323**:173-180.

Monks TJ, Jones DC, Bai F and Lau SS (2004) The role of metabolism in 3,4-(+)-methylenedioxyamphetamine and 3,4-(+)-methylenedioxymethamphetamine (ecstasy) toxicity [review]. *Ther Drug Monit* **26**:132-136.

Mueller M, Yuan J, Felim A, Neudörffer A, Peters FT, Maurer HH, McCann UD, Largeron M and Ricaurte GA (2009) Further Studies on the Role of Metabolites in MDMA-induced Serotonergic Neurotoxicity. *Drug Metab Dispos* **37**:2079-2086.

Obach RS and Reed-Hagen AE (2002) Measurement of Michaelis constants for cytochrome P450-mediated biotransformation reactions using a substrate depletion approach. *Drug Metab Dispos* **30**:831-837.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Peters FT, Samyn N, Lamers C, Riedel W, Kraemer T, de Boeck G and Maurer HH (2005) Drug Testing in Blood: Validated Negative-Ion Chemical Ionization Gas Chromatographic-Mass Spectrometric Assay for Enantioselective Determination of the Designer Drugs MDA, MDMA (Ecstasy) and MDEA and Its Application to Samples from a Controlled Study with MDMA. *Clin Chem* **51**:1811-1822.

Pizarro N, Farre M, Pujadas M, Peiro AM, Roset PN, Joglar J and de la Torre R (2004) Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). *Drug Metab Dispos* **32**:1001-1007.

Riches Z, Stanley EL, Bloomer JC and Coughtrie MW (2009) Quantitative evaluation of the expression and activity of five major sulfotransferases (SULTs) in human tissues: the SULT "pie". *Drug Metab Dispos* **37**:2255-2261.

Schwaninger AE, Meyer MR, Huestis MA and Maurer HH (2011a) Development and validation of LC-HRMS and GC-NICI-MS methods for stereoselective determination of methylenedioxymethamphetamine (MDMA) and its phase I and II metabolites in human urine. *J Mass Spectrom* **46**:603-614.

Schwaninger AE, Meyer MR, Zapp J and Maurer HH (2009) The Role of Human UDP-glucuronyltransferases on the Formation of the Methylenedioxymethamphetamine (Ecstasy) Phase II Metabolites *R*- and *S*-3-methoxymethamphetamine 4-*O*-glucuronides. *Drug Metab Dispos* **37**:2212-2220.

Schwaninger AE, Meyer MR, Zapp J and Maurer HH (2011b) Sulfation of the 3,4-methylenedioxymethamphetamine (MDMA) metabolites 3,4-dihydroxymethamphetamine (DHMA) and 4-hydroxy-3-methoxymethamphentamine (HMMA) and their capability to inhibit human sulfotransferases. *Toxicol Lett* **202**:120-128.

Shima N, Katagi M, Kamata H, Zaitsu K, Kamata T, Nishikawa M, Miki A, Tsuchihashi H, Sakuma T and Nemoto N (2008) Urinary excretion of the main metabolites of 3,4-methylenedioxymethamphetamine (MDMA), including the sulfate and glucuronide of 4-

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

hydroxy-3-methoxymethamphetamine (HMMA), in humans and rats. *Xenobiotica* **38**:314-324.

Venkatakrishnan K, von Moltke LL, Court M.H., Harmatz JS, Crespi CL and Greenblatt DJ (2000) Comparison between cytochrome P450 (CYP) content and relative activity approaches to scaling from cDNA-expressed CYPs to human liver microsomes: ratios of accessory proteins as sources of discrepancies between the approaches. *Drug Metab Dispos* **28**:1493-1504.

Wang LQ and James MO (2005) Sulfotransferase 2A1 forms estradiol-17-sulfate and celecoxib switches the dominant product from estradiol-3-sulfate to estradiol-17-sulfate. *J Steroid Biochem Mol Biol* **96**:367-374.

Youdim K and Dodia R (2010) Comparison between recombinant P450s and human liver microsomes in the determination of cytochrome P450 Michaelis-Menten constants. *Xenobiotica* **40**:235-244.

Zhang H, Varlamova O, Vargas FM, Falany CN and Leyh TS (1998) Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase. *J Biol Chem* **273**:10888-10892.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

Legends to the Figures

FIG. 1. Metabolic formation of *R*- and *S*-DHMA 3-sulfates, *R*- and *S*-DHMA 4-sulfates, and *R*-and *S*-HMMA sulfates

FIG. 2.

A: Enzyme kinetics of DHMA (left side) and HMMA (right side) sulfation by SULT1A1, SULT1A3, and SULT1E1. Data points represent means and ranges (error bars) of duplicate measurements. On the left side, the solid curves represent formation of DHMA 3-sulfate and the broken curves represent formation of DHMA 4-sulfate.

B: Plots of depletion rate constants versus substrate concentration for DHMA (left side) and HMMA (right side) sulfation by SULT1A3 and pHLC. Data points represent single measurements. The solid curves represent depletion of the *S*-enantiomers, the broken curves represent depletion of the *R*-enantiomers.

C: Correlation between K_m and V_{max} values determined using product formation and substrate depletion experiments. The broken line represents a line of unity and the solid lines represent the range of 2-fold error.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

Table 1. Kinetic Data for the Sulfation of racemic DHMA and HMMA by recombinant SULTs and pHLC

			Product Formation Approach			Substrate Depletion Approach					
			R/S			*R*			*S*		
			K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m
DHMA	SULT1A1	3-sulfate	51 ± 6	3.3 ± 0.2	0.065						
		4-sulfate	n.d.	n.d.	n.d.						
	SULT1A3	3-sulfate	0.8 ± 0.2	272 ± 17	340	0.4 ± 0.1	81 ± 17	203	0.7 ± 0.2	291± 45	416
		4-sulfate	0.5 ± 0.2	26 ± 2	52						
	pHLC	3-sulfate	1.3 ± 0.2	0.05 ± 0.002	0.038	1.3 ± 0.7	0.054 ± 0.003	0.042	1.0 ± 0.4	0.078 ± 0.007	0.078
		4-sulfate	1.6 ± 0.6	0.005 ± 0.001	0.003						
HMMA	SULT1A3		0.4 ± 0.1	410 ± 45	1025	0.4 ± 0.1	176 ± 18	440	0.4 ± 0.1	151 ± 15	378
	SULT1E1		75 ± 11	27 ± 2	0.36						
	pHLC		0.6 ± 0.1	0.16 ± 0.01	0.26	0.5 ± 0.1	0.040 ± 0.005	0.08	0.4 ± 0.1	0.035 ± 0.003	0.087

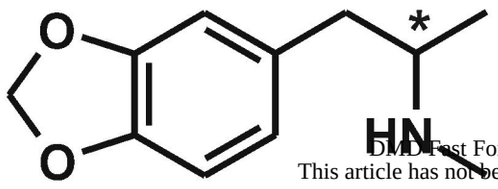
units are: K_m, µM; V_{max}, nmol/min/mg
+/- represents the error of the calculated kinetic parameters

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.

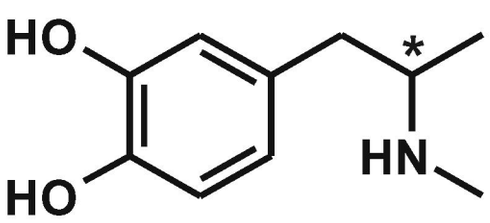
Figure 1

DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.



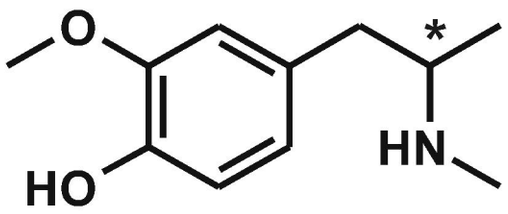
R-/*S*-MDMA

CYP



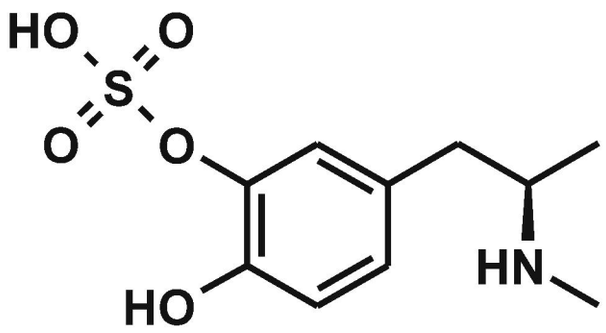
R-/*S*-DHMA

COMT

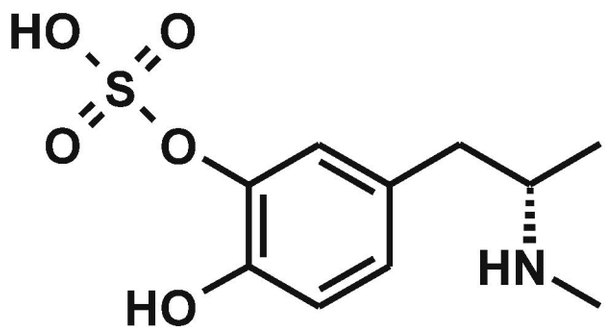


R-/*S*-HMMA

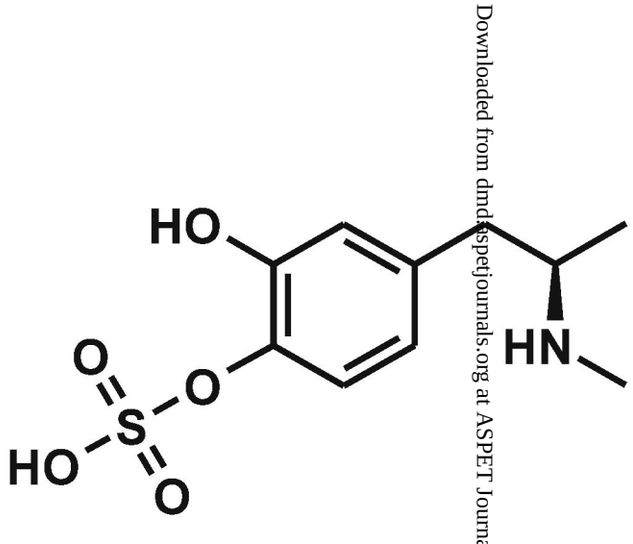
SULT



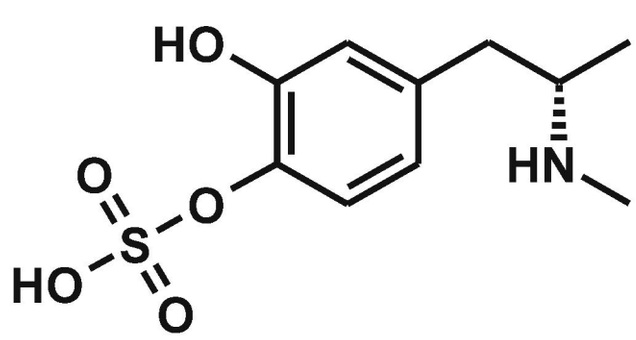
R-DHMA 3-sulfate



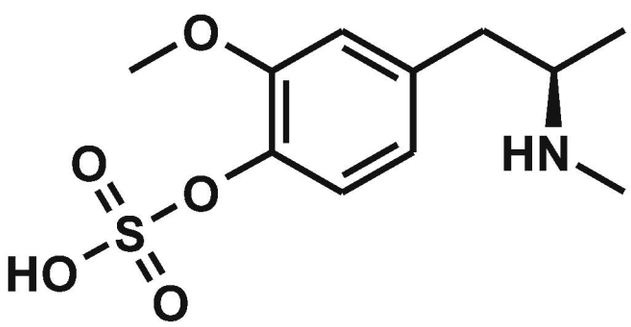
S-DHMA 3-sulfate



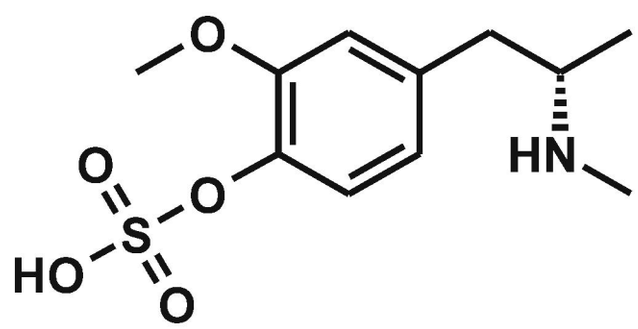
R-DHMA 4-sulfate



S-DHMA 4-sulfate



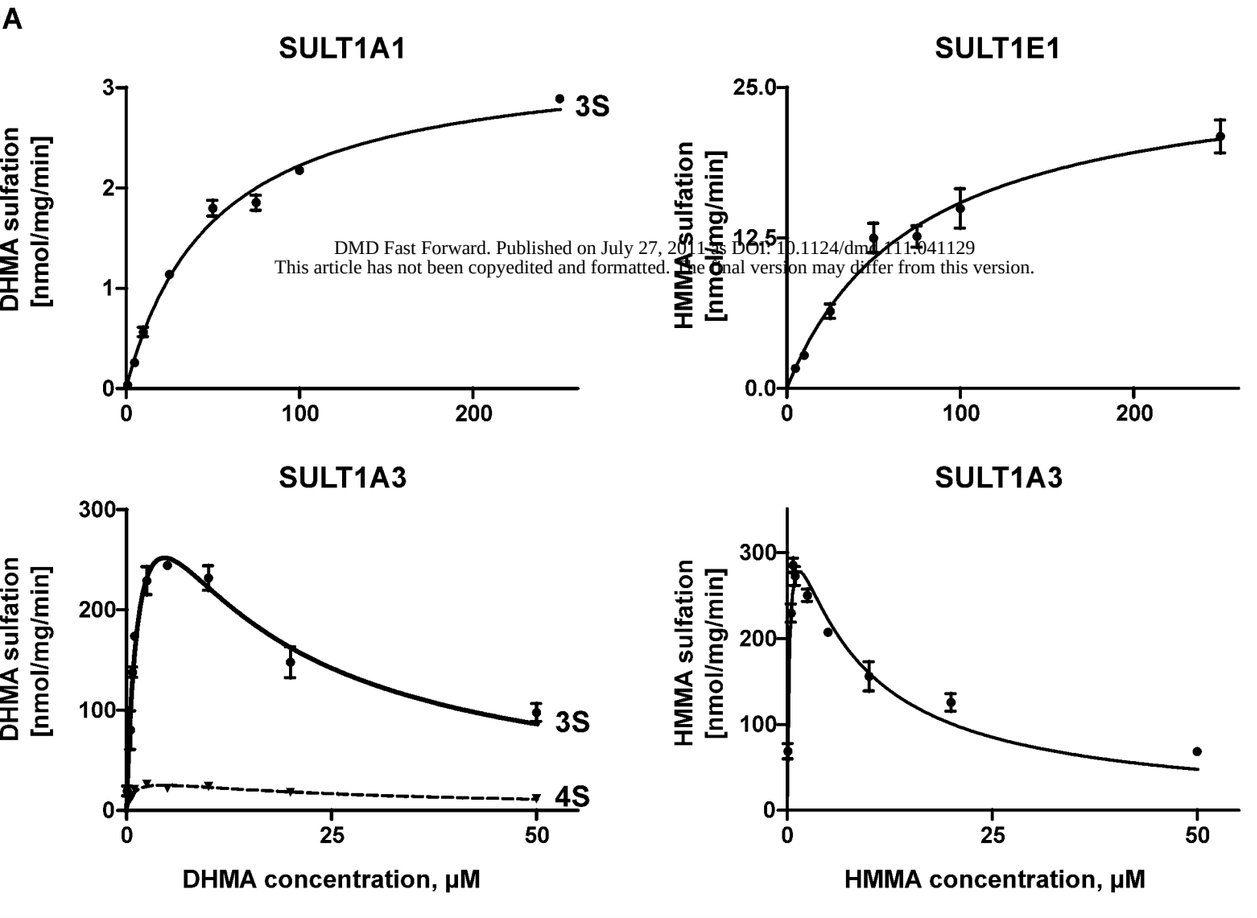
R-HMMA sulfate



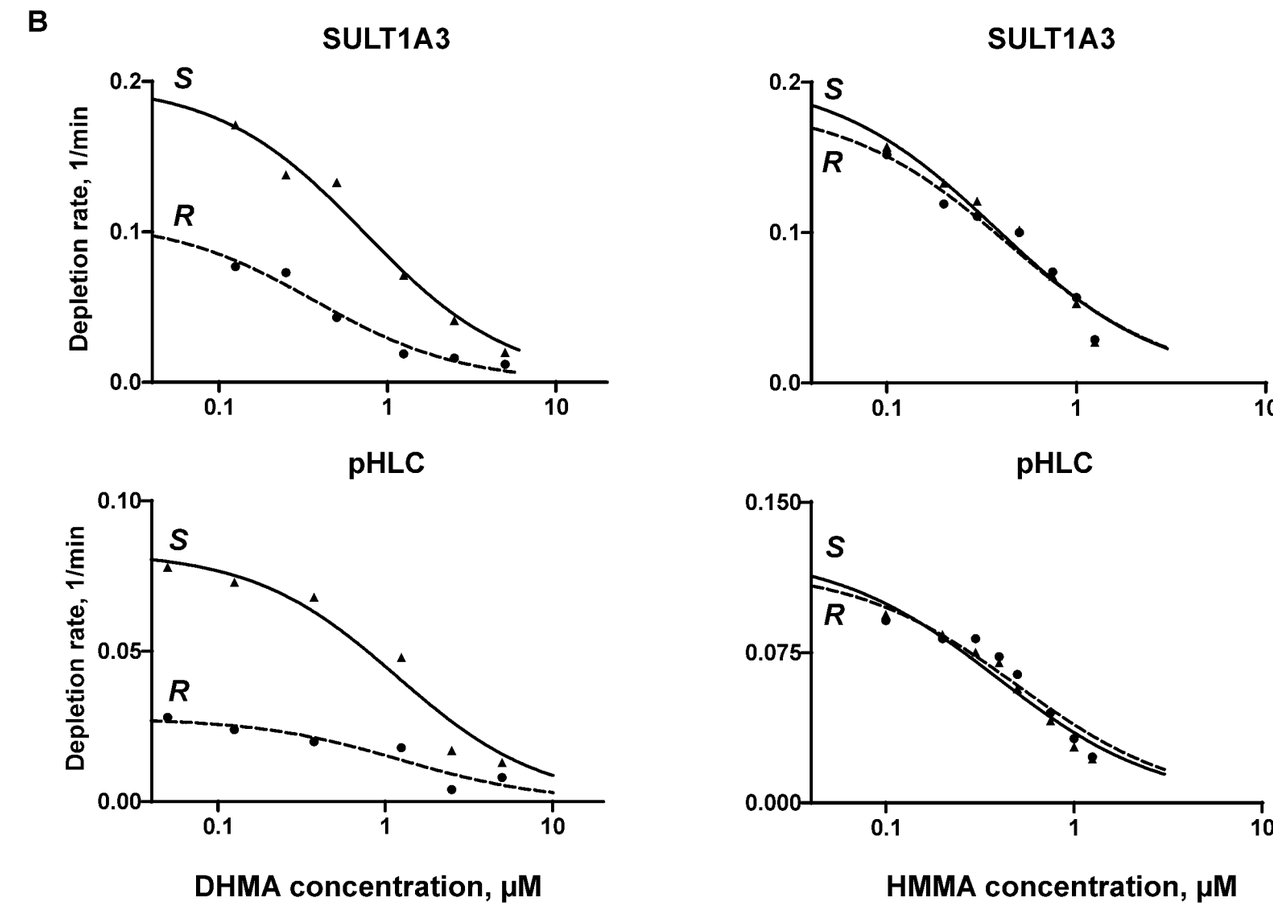
S-HMMA sulfate

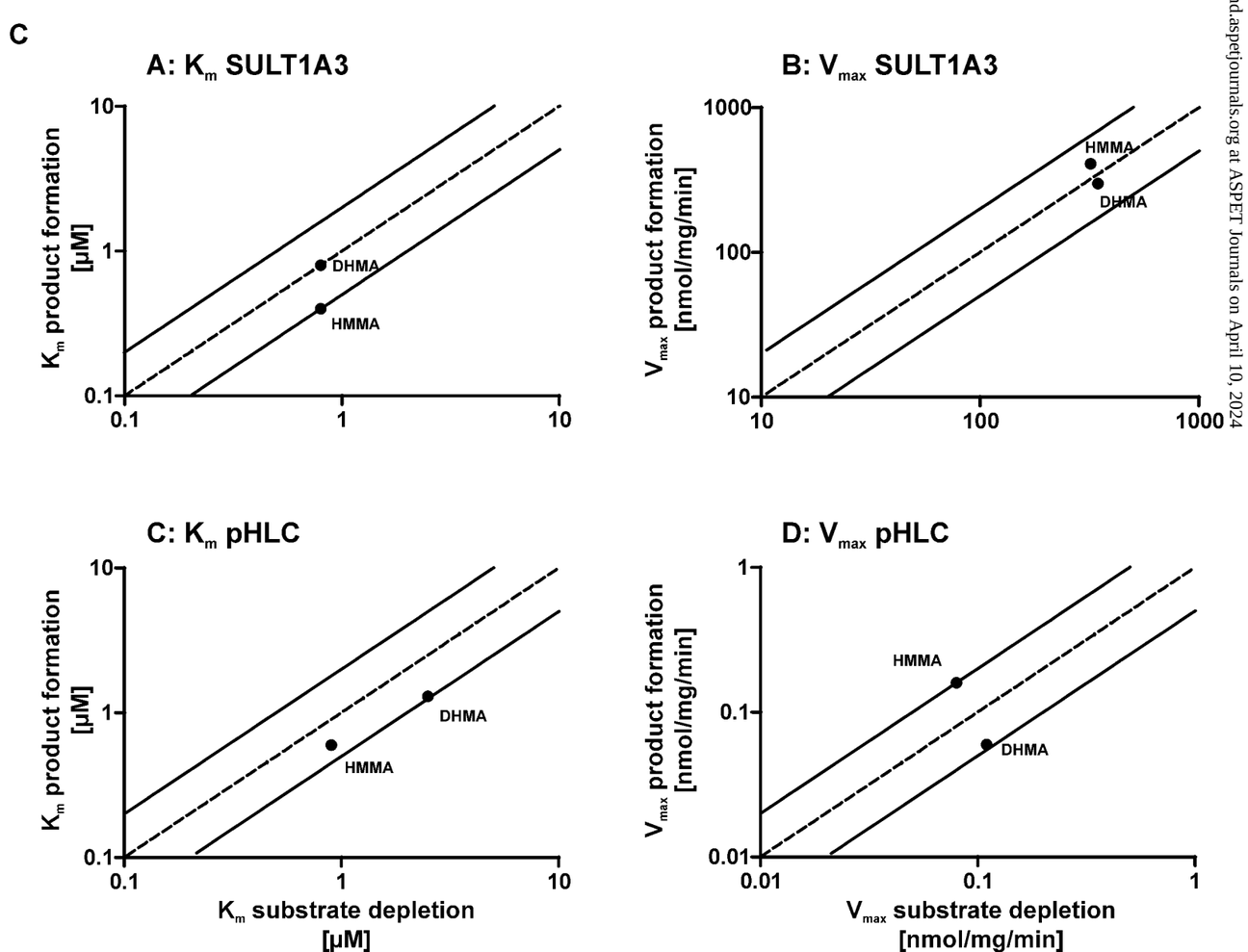
Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024

Figure 2



DMD Fast Forward. Published on July 27, 2011 as DOI: 10.1124/dmd.111.041129
This article has not been copyedited and formatted. The final version may differ from this version.





Downloaded from dmd.aspetjournals.org at ASPET Journals on April 10, 2024