Enantiomers of Methylenedioxy Designer Drugs

The Role of Cytochrome P450s and Catechol-*O*-methyltransferase in Their Metabolism

Dissertation
zur Erlangung des Grades
des Doktors der Naturwissenschaften
der Naturwissenschaftlich-Technischen Fakultät III -
Chemie, Pharmazie und Werkstoffwissenschaften
der Universität des Saarlandes

von

Markus Robert Meyer

Saarbrücken

2009

Tag des Kolloquiums:	06.08.09
Dekan:	Univ.-Prof. Dr.-Ing. S. Diebels
Berichterstatter:	Univ.-Prof. Dr. Dr. h.c. H. H. Maurer
	Univ.-Prof. Dr. R. W. Hartmann

Die folgende Arbeit entstand unter der Anleitung von Herrn Professor Dr. Dr. h.c. Hans H. Maurer in der Abteilung Experimentelle und Klinische Toxikologie der Fachrichtung 2.4 Experimentelle und Klinische Pharmakologie und Toxikologie der Universität des Saarlandes in Homburg/Saar von März 2006 bis Mai 2009.

Mein besonderer Dank gilt:

Herrn Prof. Dr. Dr. h.c. Hans H. Maurer für die herzliche Aufnahme in seinen Arbeitskreis, die Vergabe dieses interessanten und abwechslungsreichen Dissertationsthemas, die Möglichkeit des selbstständigen Arbeitens, der aktiven Teilnahme und Präsentation auf nationalen und internationalen Fachkongressen und seine Diskussionsbereitschaft zu jeder Tages- und Nachtzeit,

Herrn Prof. Dr. Rolf Hartmann für die Übernahme des Koreferats,

Herrn Dr. Frank T. Peters für seine freundschaftliche Verbundenheit, seine moralische Unterstützung (auch an Tagen sportlicher Niederlagen) und vor allem für seine außerordentliche wissenschaftliche Expertise und stete Diskussionsbereitschaft,

meinen Kolleginnen und Kollegen während der Promotionszeit für die freundschaftliche Atmosphäre, gute Zusammenarbeit und die Unterstützung in schwierigen Situationen in der Dienstbereitschaft,

Herrn Armin Weber für seine Gelassenheit, Ruhe, ständige Einsatzbereitschaft, Wartung und Reparatur der Messgeräte sowie Rat bei technischen Fragestellungen,

Frau Gabriele Ulrich und Herrn Carsten Schröder für gewissenhaft ausgeführte Laborarbeiten und Betreuung der Messgeräte,

meinem Sonnenschein für seine Wärme und Liebe,

meiner Familie, insbesondere meinen Eltern, die mich in meinem Tun jederzeit bedingungslos gefördert und auch unterstützt haben,

meinen Freunden und Bekannten, die in den letzten Jahren des Öfteren ohne mich zusammenkommen mussten und mich trotz allem nicht vergessen haben.

Für Euch!

Es ist eine Forderung der Natur, daß der Mensch mitunter betäubt werde, ohne zu schlafen; daher der Genuß in Tabakrauchen, Branntweintrinken, Opiaten.

Johann Wolfgang von Goethe, Maximen und Reflexionen

TABLE OF CONTENTS

1 GENERAL PART 1

1.1 Introduction 1

1.1.1 Designer Drugs 1

1.1.2 Metabolism 1

1.1.3 Pharmacology and Toxicology 2

1.1.4 Metabolizing Enzymes 4

1.1.4.1 Cytochrome P450 4

1.1.4.2 Catechol-O-methyltransferase 6

1.1.5 Preparation of Single Enantiomers 8

1.1.5.1 Separation of racemic MDMA, MDEA, and MBDB 8

1.1.5.2 Sample preparation and purity check using GC-MS 10

1.2 Aims and Scopes 12

2 PUBLICATIONS OF THE RESULTS 15

2.1 The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxy-methamphetamine and its enantiomers[69] (DOI: 10.1124/dmd.108.021543) 15

2.2 The Role of Human Hepatic Cytochrome P450 Isozymes in the Metabolism of Racemic MDEA and its Single Enantiomers[70] (DOI: 10.1124/dmd.108.026203) 17

2.3 Stereoselective differences in the cytochrome P450-dependent dealkylation and demethylenation of N-methyl-benzodioxolyl-butanamine (MBDB, Eden) enantiomers[71] (DOI: 10.1016/j.bcp.2009.03.001) 19

2.4 Investigations on the Human Hepatic Cytochrome P450 Isozymes Involved in the Metabolism of 3,4-Methylenedioxy-amphetamine (MDA) and Benzodioxolyl-butanamine (BDB) Enantiomers[72] (DOI: ##) 21

2.5 Enantioselectivity in the Methylation of the Catecholic Phase-I Metabolites of Methylenedioxy Designer Drugs and their Capability to Inhibit COMT Catalyzed Dopamine 3-Methylation[73] (DOI: 10.1021/tx900134e) 23

3 CONCLUSIONS 25

4 SUMMARY 27

5 REFERENCES 29

6 ABBREVIATIONS 35

7 ZUSAMMENFASSUNG 37

1 GENERAL PART

1.1 INTRODUCTION

1.1.1 Designer Drugs

Chirality and differences in pharmacodynamic and pharmacokinetic properties of the enantiomers is not restricted to legal drugs such as ketamine, omeprazole, or citalopram. Drugs of abuse can also contain chiral centers and the respective enantiomers might also differ in their properties. As consumption of drugs of abuse is a widespread problem in societies all over the world and the abused compounds are not investigated before appearance on the illicit drug market, knowledge about possible differences of their enantiomers is very limited. Especially, so-called designer drugs are more and more popular among young people. The most frequently abused drugs are amphetamine-derived designer drugs which are chiral compounds containing an asymmetric carbon atom in the side chain. One class of amphetamine-derived designer drugs are the methylenedioxy derivatives 3,4-methylene¬dioxymethamphetamine (MDMA, Ecstasy, Adam), 3,4-methylene-dioxy¬ethylamphetamine (MDEA, Eve), N-methyl-benzodioxolyl-butanamine (MBDB, Eden), 4-methylenedioxy-amphetamine (MDA), and benzodioxolyl-butanamine (BDB). Their chemical structures are shown in Fig. 1.

1.1.2 Metabolism

As shown in Fig. 1, *in vivo* studies of MDMA, MDEA, and MBDB revealed two main metabolic steps: *N*-demethylation and demethylenation. The first pathway, predominant in humans, involves cytochrome p450 (CYP)-catalyzed *O*-demethylenation to the corresponding 3,4-dihydroxy compounds followed by catechol-*O*-methyltransferase (COMT)-catalyzed *O*-methylation to the 4-hydroxy-3-methoxy metabolites and *O*-conjugation with sulfate or glucuronic acid. The second entails initial *N*-dealkylation to MDA or BDB followed by deamination and oxidation to the corresponding benzoic acid derivatives conjugated with glycine.[1-3] Urinary recovery of MDMA represents about 15% which indicates that the drug is mainly

eliminated by metabolism.[3] MDA and BDB can also undergo *O*-demethylenation to the 3,4-dihydroxy compounds following *O*-methylation and *O*-conjugation with glucuronic acid or sulfate. The catechols, formed via metabolic demethylenation of the aforementioned drugs, can easily be oxidized to their corresponding ortho-quinones which in turn can form adducts with glutathione and other thiol-containing compounds.[4,5]

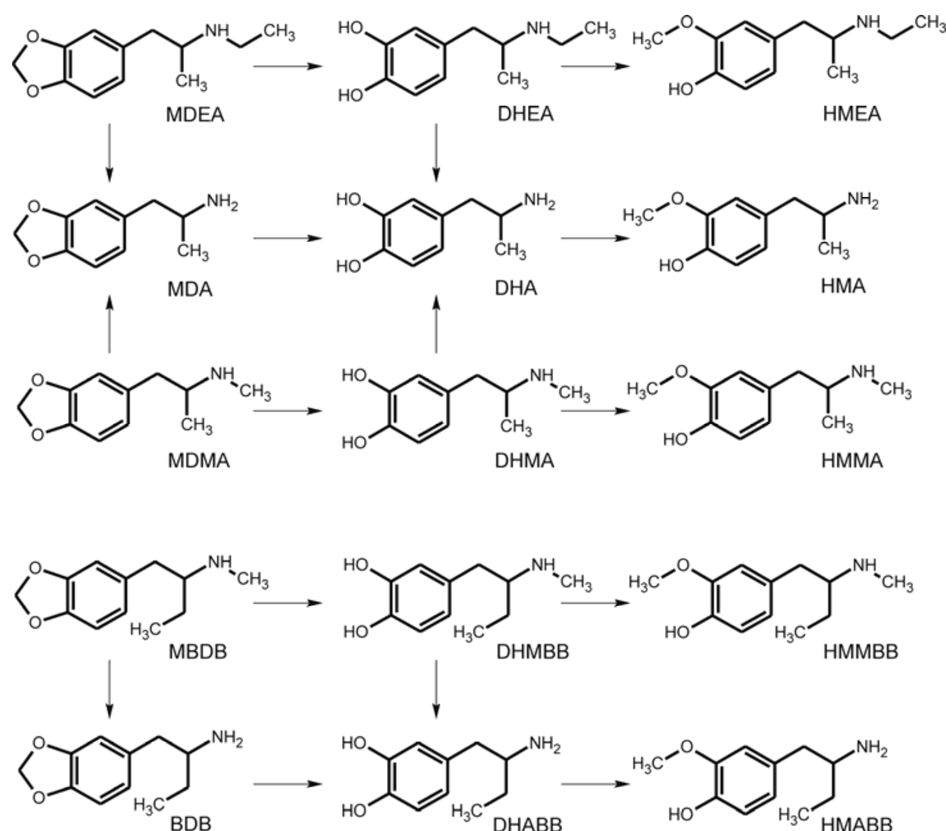

Fig. 1: Chemical structures and main metabolic steps of the studied amphetamine-derived designer drugs

1.1.3 Pharmacology and Toxicology

MDMA, MDEA, and MBDB have effects on the central nervous system described as altered state of consciousness, well being, increased tactile sensations, and a strong desire to socialize.[6,7] Additionally, they can increase the concentration of dopamine, serotonin, and noradrenaline in the central nervous system. MDA itself is reported to be twice as potent as MDMA and MDEA.[7] However, BDB should have similar

pharmacological properties as the related aforementioned compounds. Therefore, they are all members of the same drug class called “entactogens”.[8]

Different pharmacokinetic properties were described for MDMA, MDEA, and MDA enantiomers.[9-14] Johnson et al. reported about differences in the dose response curve and for changes in serotonergic function and neurotoxicity between *S*-(+)-MDA and *R*-(-)-MDA.[15] Several studies have shown that there is a metabolic preference for the *S*-enantiomer of MDMA.[9-14] MDEA was investigated concerning enantioselective pharmacokinetics *in vivo*[16,17] and the plasma half life of *R*-MDEA was found to be longer than that of *S*-MDEA. Accordingly, the plasma concentrations of the *S*-enantiomers of the main metabolites *N*-ethyl-4-hydroxy-3-methoxyamphetamine (HMEA) and MDA were much higher than those of the *R*-enantiomers. Enantioselective pharmacokinetics of MDEA resulting in higher plasma concentrations of *R*-MDEA were also confirmed by other authors.[12,18,19]

Concerning chronic toxicity, data strongly suggest that adducts of the catecholic metabolites of MDMA, MDEA, MBDB, and MDA can cause irreversible damage to serotonergic nerve terminals in the central nervous system and systemic metabolism may play a role in their neurotoxicity.[3,9,20-27] This was concluded from the observation that direct injection of ecstasy into the brain fails to reproduce the neurotoxic effects seen after systemic administration,[28] and from the report that alteration of CYP-mediated MDMA metabolism influences MDMA induced neurotoxicity.[28,29] Metabolites such as DHMA are easily be oxidized to their corresponding quinones which can form adducts with glutathione and other thiol-containing compounds.[4,30,31] Recently, such adducts have been implicated in MDMA neurotoxicity.[32,33] Only few publications are available on the neurotoxicity in living humans. In these studies, recreational MDMA users were found to have decreased levels of 5-hydroxyindoleacetic acid, the main metabolite of serotonin, in the cerebrospinal fluid[34] and a reduced density of serotonin transporters in the brain as determined by positron emission computed tomography with a ligand selective for these transporters.[35] Both findings are indicative of serotonin neurotoxicity in humans. Unfortunately, these studies were performed with recreational users, so it cannot be excluded whether the reported findings might also be due to use of other recreational drugs especially since polydrug use is not uncommon.

1.1.4 Metabolizing Enzymes

1.1.4.1 Cytochrome P450

In general, drugs are metabolized by a variety of enzymes, producing metabolites that are usually less toxic than their parent compounds. The formed metabolites may also be more reactive, producing toxic effects. CYP enzymes are responsible for oxidative and, to a minor extent, reductive metabolic transformations of drugs, environmental chemicals and natural compounds. Over its long history of more than 3.5 billion years, the CYP superfamily of enzymes has developed remarkable versatility. As shown in figure 2, the primary catalytic function of CYPs was identified as transfer of one oxygen atom from molecular oxygen into various substrates. A coenzyme, cytochrome P450 oxidoreductase (OR), is essential for CYP catalytic function, and cytochrome b_5 can stimulate catalytic activities of some enzymes.[36]

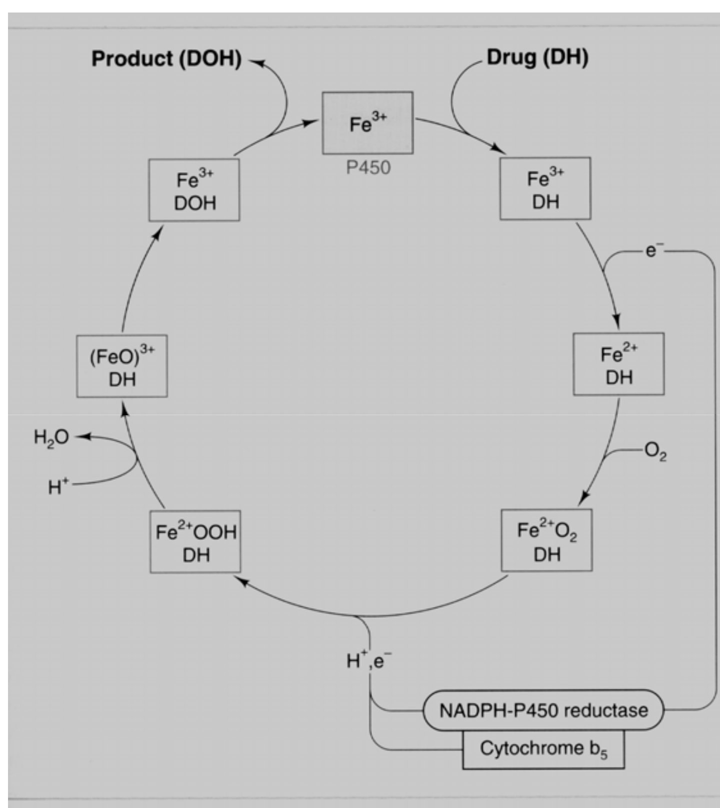

Fig. 2: The cytochrome P450 redox cycle.

Single electron shifts are frequently responsible for the formation of reactive intermediates or allow the leakage of free radicals capable of causing toxicity. When

a CYP enzyme activity is modified by induction or inhibition, the biological activity of the xenobiotic substrate can be altered considerably. Such effects are called drug-drug, drug-chemical or chemical-chemical-interactions. Such interactions can modify the disposition of xenobiotics.[37-39] In mammals, the enzymes can be identified in nearly every tissue, being most abundantly present in the liver. The CYP superfamily has been classified in different families in accordance to the degree of homology of amino acid sequence in their protein structures. CYP enzymes having $\leq$ 40% homology in their amino acid sequence are classified in different families which are designated by Arabic numbers, for example, CYP1. Each family is further divided into subfamilies of enzymes. The enzymes within a mammalian subfamily have $\geq$ 55% sequence homology and are designated by capital letters, for example, CYP1A. An Arabic number is used for designating individual enzymes within a subfamily, for example, CYP1A2.[37] In humans, 18 CYP families with 43 subfamilies and 57 CYP isoenzymes are known so far, of which only 3 families with 7 subfamilies and 12 CYP isoenzymes are relevant for drug metabolism,[40] namely CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5.[41]

Human liver-derived enzyme preparations, e.g. human liver microsomes (HLM) contain a natural mixture of CYPs. Chemical inhibitors, immunochemical inhibitors, and/or correlation analyses with marker activities must be used to obtain information on which enzymes are performing specific biotransformations. In contrast, only a single active CYP is present in preparations of cDNA-expressed enzymes. Inhibitors and correlation analyses are not needed, because the mentioned assignments can be performed by direct incubation of the drug with a panel of individual CYPs. However, the balance of enzymes, present in vivo, is lost.[36] Bacteria, yeast, baculovirus and several mammalian cells have been used to produce a wide range of catalytically active CYPs. The baculovirus system offers high-level expression of both the CYP and OR, and are therefore advantageous for metabolism studies of all kinds, especially for low turnover substrates. The development of the cDNA-bearing virus is relatively time-consuming and labor-intensive, but baculovirus infected insect cell microsomes are commercially available. However, because the enzymes are produced transiently in the insect host cells, exact harvest time can have a pronounced effect on the activity of the final preparation.[42]

1.1.4.2 Catechol-O-methyltransferase

Julius Axelrod, an American biochemist (1912-2004), whose Nobel Prize-winning research grew out of work done by Euler, specifically Euler's discovery of noradrenaline (norepinephrine). Axelrod, in turn, discovered that noradrenaline could be neutralized by an enzyme, catechol-*O*-methyltransferase (COMT, EC 2.1.1.6), which he isolated and named.

In either rats or humans, there is only one single gene for COMT, encoding the soluble COMT (S-COMT) and the membrane-bound COMT (MB-COMT) by using two separate promoters.[43,44] In most human tissues, the majority of COMT is present in the soluble form (S-COMT). However, in human brain, 70% of the total COMT proteins was found to be MB-COMT and 30% of them S-COMT. Analyses with overexpressed MB-COMT showed that it is mainly located in the rough endoplasmic reticulum, facing the cytoplasm, and no MB-COMT is present in the cytoplasmic membrane. The overexpressed S-COMT in cultured cells was found to be localized in cytosol and nucleus.[45,46]

These enzymes proved critical to an understanding of the entire nervous system because they catalyze the enzymatic *O*-methylation of endogenous catecholamines and other catechols.[47] The physiological substrates of COMT include catecholamines (dopamine, norepinephrine, and epinephrine), catechol estrogens, and many others. In addition, many drugs, such as dobutamine, isoprenaline, levodopa, and benserazide are also substrates of COMT.[48-51] A few recent studies demonstrated that several dietary phytochemicals such as bioflavonoids and tea catechins are exceptionally good substrates for the COMT-mediated *O*-methylation with metabolic rates much higher than endogenous catecholamines and catechol estrogens.[52-55] COMT has also been suggested to have additional important physiological functions in organs like the kidney and intestine through modulating the rate of dopamine metabolism which should also be true in the brain. COMT may modulate the neurotransmitter functions of dopamine and norepinephrine altering the rate of their metabolic inactivation.[56]

As depicted in Fig. (3), COMT catalyzes the transfer of the methyl group from *S*-adenosylmethionine (SAM) to one of the two hydroxyl groups of the catechol substrates in the presence of Mg^{2+} as a cofactor.[49]

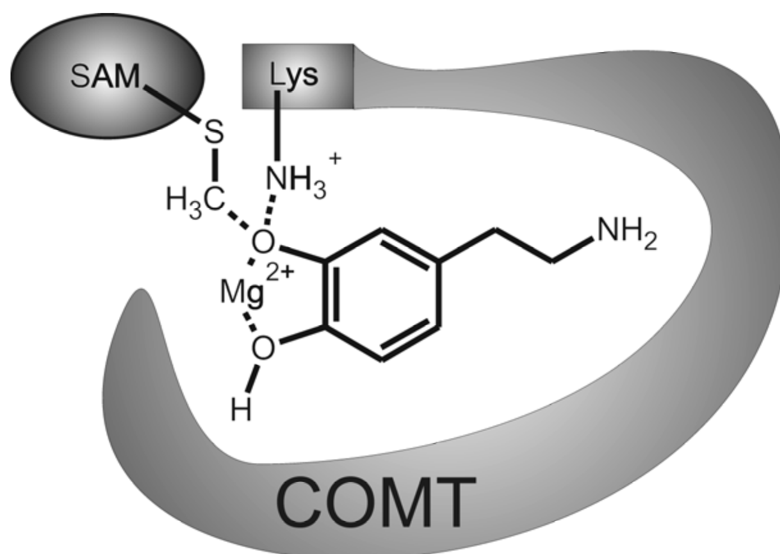

Fig. 3: Schematic illustration of the catalytic mechanism of S-COMT-mediated O-methylation. The dotted lines indicate the possible non covalent interactions between the molecules or atoms.

The binding of Mg^{2+} to the COMT protein improves the ionization of the two hydroxyl groups of the catechol substrate. The lysine residue (Lys144) accepts the proton of one of the two hydroxyl groups, acting as a catalytic base for the nucleophilic methyl transfer reaction. An earlier study suggested that the methyl transfer proceeds through a direct nucleophilic attack by one of the hydroxyl groups of the catechol substrate at the methyl carbon of SAM in a tight *SN2*-like transition state.[57,58] Nevertheless, there are also a number of *S*-COMT and *MB*-COMT characteristics for the *O*-methylation of different catechols *in vitro*. First, *S*-COMT generally has much higher apparent K_M values (lower affinity) for various substrates than *MB*-COMT (higher affinity).[59,60] Second, despite the generally low affinities of *S*-COMT for various substrates, its overall catalytic capacity (V_{max}) is ten to one hundred folds higher than values for the MB-COMT. Third, for the *O*-methylation of catecholamines, both *S*-COMT and *MB*-COMT favor 3-*O*-methylation (*meta*-position) over 4-*O*-methylation (*para*-position). It appears that *MB*-COMT is even more regioselective than *S*-COMT in favor of 3-*O*-methylation.[61] The reason for the favorable 3-*O*-methylation over 4-*O*-methylation may be as follows. While the 4-*O*-hydroxyl group of the substrate approaches SAM, its side chain is forced to be orientated in an unfavorable position towards a cluster of hydrophobic amino acid residues located at or near the catalytic site (see Fig. 3). Molecular dynamic simulation studies appeared to be in agreement with this explanation.[62,63]

Val158Met is one single nucleotide polymorphism in the gene that codes COMT. This single nucleotide substitution between G and A results in an amino acid change from valine to methionine at codon 158 and provides a higher activity of the enzyme. It was recently associated with modulation of cognition and diseases like schizophrenia.[64]

Characterization of the human enzymes involved in the metabolism of specific drugs is becoming increasingly important. Such characterization should consider two processes involving the new drug: metabolism and inhibition. The characterization of enzymes involved in metabolism of a new drug allows prediction, based on knowledge of the ability of co-administered drugs to inhibit the same enzymes, of which co-administered drugs may inhibit the metabolism of the new drug. This information can also be used to predict individual variability based on known metabolic polymorphisms.[36] However, also the new drug can act as an inhibitor what may lead to interactions with co-administered drugs.

1.1.5 Preparation of Single Enantiomers

1.1.5.1 Separation of racemic MDMA, MDEA, and MBDB

MDMA, MDEA, and MBDB were separated using a Hewlett Packard Series 1050 semi-preparative HPLC system consisting of a pump and a variable wavelength detector (λ = 263 nm for MDMA and MDEA and λ = 285 nm for MBDB) which was coupled to an Advantec SF 2120 Super Fraction collector. The stationary phase was a Merck Hibar HPLC ChiraDex column (250 x 10 mm, 5 µm). The mobile phase varied in dependence of the separated racemic mixture to obtain best separation. The respective conditions are given in Table 1. Analytes were separated in aliquots (100 µl, MDMA and MDEA; 250 µl, MBDB) of an aqueous stock solution (5 mg/ml MDMA and MDEA; 1 mg/ml, MBDB). In total, 40 mg MDMA, 30 mg MDEA, and 50 mg MBDB were separated. Fractions were collected and checked for optical purity. Therefore, 10 µl of the respective fraction was transferred to 1.5 ml reaction caps and diluted with 200 µl aqueous carbonate buffer (35 g/l sodium bicarbonate and 15 g/l sodium carbonate, pH 9). Purity was checked by gas chromatography-mass spectrometry (GC-MS) as described in detail under sample preparation for purity check.

	mobile phase A	mobile phase B	flow rate	pH and temperature
R,S-MDMA	0.1 M ammonium acetate buffer 85%	acetonitrile 15%	3 ml/min	pH 6.5 at 8°C
***R,S*-MDEA**	0.1 M ammonium acetate buffer 85%	acetonitrile 15%	3 ml/min	pH 6.5 at 8°C
***R,S*-MBDB**	0.1 M ammonium acetate buffer 85%	15% mobile phase B (acetonitrile 49.5%, methanol 49.5%, and triethylamine 1%),	2 ml/min	pH 7.0 at 15°C

Tab. 1: HPLC conditions for separation of the racemic mixtures.

For isolation of MDMA enantiomers from the collected fractions, the acetonitrile part of the HPLC solvent was evaporated. The remaining part was acidified with 1 ml 0.01 mol/L HCl and the enantiomers were isolated from the aqueous part by solid phase extraction (SPE) using Varian Bond Elut SCX HF cartridges (5 g, 20 ml), previously conditioned with 10 ml of methanol and 10 ml of water. After passage of the fractions, the cartridges were washed with 10 ml of 0.01 mol/L hydrochloric acid and 10 ml of methanol. The compounds were eluted twice with 10 ml freshly prepared mixture of methanol/aqueous ammonia (96:4 v/v). The eluates were evaporated to dryness under reduced pressure and reconstituted in 1.0 ml 0.01 mol/L HCl and quantified according to ref.[13] Forty mg of racemic MDMA-HCl were separated under the described conditions.

For MDEA, the fractions containing the separated enantiomers were collected and the enantiomers were isolated from the aqueous part by liquid/liquid extraction at pH 9 using ethyl acetate (three times using 150 ml each). The extracts were evaporated to dryness using a Rotavapor under reduced pressure and reconstituted in 1.0 ml of 0.01 M HCl. Thereafter, the concentrations of the MDEA enantiomers in the resulting solution were determined according to the sample preparation and purity check section.

For MBDB, the respective enantiomer fractions were adjusted to pH 12 with sodium hydroxide and extracted three times with ethyl acetate (150 mL). The combined extracts were dried using magnesium sulfate. Afterwards they were concentrated to a volume of approximately 1 mL under reduced pressure. Finally, the amount and

purity of the MBDB enantiomers were checked as described in detail under sample preparation. Fifty mg of racemic MBDB were separated under the described conditions. As the chiral HPLC method did not provide a satisfying purity and separation of the enantiomers, the above described separation and extraction procedure was performed twice.

Various buffers and buffer concentrations as well as organic modifiers were tested for semi-preparative isolation of the single enantiomers for these experiments. The best result in enantiomer separation was achieved with KH_2PO_4 0.1 mol/L/acetonitrile 95/5 with triethylamine (TEA) 0.1% but the TEA content as well as the phosphate buffer turned out to be problematic in the following isolation of the enantiomers by SPE. The conditions described above were finally preferred because they yielded sufficient separation combined with favorable properties for further workup. The final products were obtained as yellowish powders or aqueous solution of high optical purities. Despite rather low recoveries (MDMA ~ 60%, MDEA ~ 75%, MBDB ~ 40% per enantiomer), the isolated amounts were sufficient for further kinetic studies.

1.1.5.2 Sample preparation and purity check using GC-MS

Derivatization was performed according to Peters et al.,[13] with slight modifications: after adding 20 µl derivatization reagent (0.1 mol/l *S*-HFBPCl in dichloromethane), the reaction vials were sealed and left on a rotary shaker at ambient temperature for 30 min. After addition of 100 µl cyclohexane to the reaction vials, they were resealed, and placed on a rotary shaker for 5 min. After phase separation by centrifugation (10000 *g* for 1 min), the cyclohexane phase was transferred to autosampler vials. Aliquots of 3 µl were injected into the GC-MS.

The samples were analyzed by an Agilent Technologies (AT) 6890 Series GC system combined with an AT 5973 network mass selective detector, an AT 7683 series injector, and an AT enhanced Chem Station G1701CA, version C.00.00 21-Dec-1999. For detection of MDMA, MDEA, MBDB enantiomers and the internal standard MDA-d_5, the GC conditions were as follows: splitless injection mode; column, 5% phenyl methyl siloxane (HP-5MS; 30 m x 0.25 mm (i.d.); 250 nm film thickness); injection port temperature, 280°C; carrier gas, helium; flow rate, 1 ml/min; column temperature.

For separation and detection of MDMA, MDEA, and MBDB the oven and MS conditions were as follows:

100°C increased to 200°C at 30°C/min, to 260°C at 5°C/min, and to 310°C at 30°C/min. The negative-ion chemical ionization (NICI)-MS conditions were as follows: transfer line heater, 280°C; NICI, methane (2 ml/min); source temperature, 150°C; solvent delay, 11 min; selected-ion monitoring (SIM) mode with the following ions: *m/z* 432 for MDA-d_5 *m/z* 446 for MDMA and 460 for MDEA and MBDB. For separation of MBDB, the GC conditions were modified as follows: 100°C increased to 221°C at 50°C/min, to 240°C at 4°C/min, and to 310°C at 50°C/min. Solvent delay, 2 min; Enantiomers were quantified by comparison of their peak-area ratios (enantiomers of analyte vs corresponding enantiomer of the IS) to calibration curves in which the peak-area ratios of enriched calibrators had been plotted vs their concentrations using a weighted (1/*x*) least-squares linear regression model.

1.2 Aims and Scopes

Some ring substituted amphetamines (RSA) were shown to be metabolized (*in vitro* and *in vivo)* more or less enantioselectively.[9-14] Therefore, elucidating this phenomenon is important from the toxicological and pharmacological point of view. Concerning the most popular RSA MDMA, several studies have shown that there is a metabolic preference for the *S*-enantiomer[9-14] and that this difference might be attributable to cytochrome P450 CYP2D6.[65-67] MDEA was also investigated concerning enantioselective pharmacokinetics *in vivo*[16,17] and the plasma half life of *R*-MDEA was found to be longer than that of S-MDEA. In the case of MBDB, the data currently available give no idea whether the S-form of this RSA is also metabolized preferably by CYP isoforms. Neither systematic *in vivo* nor *in vitro* studies are available concerning the CYP-dependent metabolism of MDMA, MDEA, and MBDB enantiomers with respect to all relevant isoforms. Only studies using racemic mixtures and CYP inhibition in pooled human liver microsomes are available.[2] In the case of MDA and BDB, the data currently available provide no information on whether the *S*-forms of these RSA are also metabolized preferably by CYP isoforms. Neither *in vivo* nor *in vitro* studies are available concerning the CYP-dependent metabolism of their enantiomers. In addition, it should be of interest to see whether theses compounds, considered as MDMA, MDEA, or MBDB metabolites, are eliminated enantioselectively.

Additionally, the question arose whether the primary catecholic metabolites of MDMA, MDEA, and MBDB are further methylated with a preference for the *S*-form. This might help to further explain the pharmacokinetic difference between the respective enantiomers and whether there are differences in the catalytic behavior between S-COMT and MB-COMT. Besides this, an inhibition potential of the catechols towards the COMT should be checked as it is known, that MDMA is a mechanism based inhibitor of CYP2D6.[68]

Therefore, the aims of the presented studies were:

- To obtain enantioselective enzyme kinetic data of MDMA, MBDB, and MDEA demethylenation and dealkylation by the ten CYPs most relevant in human drug metabolism

- To obtain enantioselective enzyme kinetic data of the demethylenation of their metabolites MDA and BDB

- To obtain enantioselective enzyme kinetic data of the methylation of their metabolites DHMA, DHEA, and DHMBB

- Determination of the inhibition potential of DHMA, DHEA, and DHMBB on COMT

2 PUBLICATIONS OF THE RESULTS

The results of the studies were published in the following papers:

2.1 THE ROLE OF HUMAN HEPATIC CYTOCHROME P450 ISOZYMES IN THE METABOLISM OF RACEMIC 3,4-METHYLENEDIOXY-METHAMPHETAMINE AND ITS ENANTIOMERS[69]

(DOI: 10.1124/DMD.108.021543)

2.2 THE ROLE OF HUMAN HEPATIC CYTOCHROME P450 ISOZYMES IN THE METABOLISM OF RACEMIC MDEA AND ITS SINGLE ENANTIOMERS[70] (DOI: 10.1124/DMD.108.026203)

2.3 Stereoselective differences in the cytochrome P450-dependent dealkylation and demethylenation of N-methyl-benzodioxolyl-butanamine (MBDB, Eden) enantiomers[71]

(DOI: 10.1016/j.bcp.2009.03.001)

2.4 INVESTIGATIONS ON THE HUMAN HEPATIC CYTOCHROME P450 ISOZYMES INVOLVED IN THE METABOLISM OF 3,4-METHYLENEDIOXY-AMPHETAMINE (MDA) AND BENZODIOXOLYL-BUTANAMINE (BDB) ENANTIOMERS[72] (DOI: 10.1016/J.TOXLET.2009.06.866)

2.5 Enantioselectivity in the Methylation of the Catecholic Phase-I Metabolites of Methylenedioxy Designer Drugs and their Capability to Inhibit COMT Catalyzed Dopamine 3-Methylation[73] (DOI: 10.1021/tx900134e)

3 CONCLUSIONS

The studies presented here provided systematic data on the involvement of cytochrome P450 isozymes in the metabolism of methylenedioxy designer drugs MDMA (Ecstasy), MBDB (Eden), and MDEA (Eve) with respect to their chirality. Additionally, it was shown that these drugs are *N*-dealkylated and demethylenated enantioselectively with a preference for the *S*-enantiomer.[69-71] Differences in the enantioselectivity of the involved isoforms where observed, indicating CYP2C19 to be the most selective in all cases. Furthermore, their *N*-dealkyl metabolites MDA, BDB are also demethylenated with a preference for their *S*-enantiomers, but these primary amines are not metabolized as enantioselectively as the secondary amines.[72] The catecholic phase-I metabolites of the aforementioned designer drugs were investigated for enantioselective methylation and their *S*-enantiomer were also shown to be preferablely formed.[73] Inhibition studies performed with DHMA, DHEA, and DHMBB clearly indicated an uncompetitive inhibition of the COMT catalyzed *O*-methylation of dopamine.[73]

These findings must be considered when trying to estimate the time of ingestion from drug enantiomer ratios in plasma[10,12] because the time course of such ratios might be considerably different in CYP2D6 poor metabolizers or in case of inhibition of CYP2D6 by co-ingested drugs. In addition, it must be considered that correlation of the presented *in vitro* data with the *in vivo* situation is not straightforward, because *in vivo* the formed metabolites are further metabolized by *O*-methylation and/or glucuronidation/sulfation. Enantioselectivity of these phase II reactions might of course also influence the enantiomer ratios in plasma samples. Considering these findings along with the fact that demethylenation is the major metabolic step *in vivo*, the different pharmacokinetic properties of the enantiomers are therefore most likely attributable to enantioselective demethylenation by CYP2C19, CYP2D6, and CYP3A4. CYP2D6 should be most important in this context, because it is the most abundant concerning net clearance at plasma concentrations observed in recreational users.

Concerning the catecholic metabolites, in the author's opinion, a part of the described neurotoxicity of the methylenedioxy designer drugs[3,9,20,26,27] could be explained by inhibition of the dopamine methylation in the central nervous system (CNS). As

MDMA and related drugs are able to increase the concentration of dopamine and other neurotransmitters in the CNS[8] and as they additionally could inhibit the inactivation of these compounds, the described dopamine induced neurotoxicity might be enhanced.[74] This might be one reason for the drug-induced irreversible damage to central nerve terminals.

4 SUMMARY

In the presented studies, the CYP dependent, enantioselective *N*-dealkylation and demethylenation of the designer drugs MDMA (Ecstasy), MBDB (Eden), and MDEA (Eve) was investigated. Furthermore, the COMT-catalyzed *O*-methylation of the supposed neurotoxic catecholic metabolites of the aforementioned drugs and the demethylenation of the dealkyl metabolites MDA and BDB was studied. The data clearly indicated a metabolic preference for the *S*-enantiomer of all investigated compounds, indicating CYP2C19 to be the most selective in all cases. Furthermore, their *N*-dealkyl-metabolites MDA, BDB are also demethylenated with a preference for their *S*-enantiomers. Data also suggest that the primary amines are not metabolized as enantioselectively as the secondary amines. The catecholic phase-I metabolites are also enantioselectively methylated with a preference for their *S*-enantiomer. These findings explain in part the observed different *in vivo* kinetic of these methylenedioxy designer drugs. Inhibition studies with the catecholic phase-I metabolites DHMA, DHEA, and DHMBB indicated an uncompetitive inhibition of the sCOMT catalyzed dopamine 3-methylation. This inhibition of the dopamine methylation in the central nervous system could be another reason for the drug-induced irreversible damage to central nerve terminals.

5 REFERENCES

1. Maurer HH. On the metabolism and the toxicological analysis of methylenedioxyphenylalkylamine designer drugs by gas chromatography-mass spectrometry. *Ther. Drug Monit.* 1996; **18**: 465.
2. Maurer HH, Bickeboeller-Friedrich J, Kraemer T, Peters FT. Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ("Ecstasy"). *Toxicol. Lett.* 2000; **112**: 133.
3. de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M, Segura J, Cami J. Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition [review]. *Ther. Drug Monit.* 2004; **26**: 137.
4. Hiramatsu M, Kumagai Y, Unger SE, Cho AK. Metabolism of methylenedioxymethamphetamine: formation of dihydroxymethamphetamine and a quinone identified as its glutathione adduct. *J. Pharmacol. Exp. Ther.* 1990; **254**: 521.
5. Miller RT, Lau SS, Monks TJ. Metabolism of 5-(glutathion-S-yl)-alpha-methyldopamine following intracerebroventricular administration to male Sprague-Dawley rats. *Chem. Res. Toxicol.* 1995; **8**: 634.
6. Nichols DE, Hoffman AJ, Oberlender RA, Jacob P, Shulgin AT. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: representatives of a novel therapeutic class. *J. Med. Chem.* 1986; **29**: 2009.
7. Hegadoren KM, Baker GB, Bourin M. 3,4-Methylenedioxy analogues of amphetamine: defining the risks to humans [review]. *Neurosci. Biobehav. Rev.* 1999; **23**: 539.
8. Nichols DE. Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. *J. Psychoactive Drugs* 1986; **18**: 305.
9. Kalant H. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs [review]. *Can. Med. Assoc. J.* 2001; **165**: 917.
10. Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA, Hutt AJ. Stereospecific analysis and enantiomeric disposition of 3, 4-methylenedioxymethamphetamine (Ecstasy) in humans. *Clin. Chem.* 1999; **45**: 1058.
11. Kraemer T, Maurer HH. Toxicokinetics of amphetamines: Metabolism and toxicokinetic data of designer drugs, of amphetamine, methamphetamine and their N-alkyl derivatives [review]. *Ther. Drug Monit.* 2002; **24**: 277.
12. Peters FT, Samyn N, Wahl M, Kraemer T, de Boeck G, Maurer HH. Concentrations and Ratios of Amphetamine, Methamphetamine, MDA, MDMA, and MDEA Enantiomers determined in Plasma Samples From Clinical Toxicology and Driving Under the Influence of Drugs Cases by GC-NICI-MS. *J. Anal. Toxicol.* 2003; **27**: 552.
13. Peters FT, Samyn N, Lamers C, Riedel W, Kraemer T, de Boeck G, Maurer HH. 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.* 2005; **51**: 1811.
14. Pizarro N, Farre M, Pujadas M, Peiro AM, Roset PN, Joglar J, de la Torre R. Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). *Drug Metab. Dispos.* 2004; **32**: 1001.
15. Johnson M, Letter AA, Merchant K, Hanson GR, Gibb JW. Effects of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine isomers on central serotonergic, dopaminergic and nigral neurotensin systems of the rat. *J. Pharmacol. Exp. Ther.* 1988; **244**: 977.
16. Brunnenberg M, Kovar KA. Stereospecific analysis of ecstasy-like N-ethyl-3,4-methylenedioxyamphetamine and its metabolites in humans. *J. Chromatogr. B Biomed. Sci. Appl.* 2001; **751**: 9.

17. Buechler J, Schwab M, Mikus G, Fischer B, Hermle L, Marx C, Gron G, Spitzer M, Kovar KA. Enantioselective quantitation of the ecstasy compound (R)- and (S)-N-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma and urine. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2003; **793**: 207.
18. Meyer A, Mayerhofer A, Kovar KA, Schmidt WJ. Enantioselective metabolism of the designer drugs 3,4-methylenedioxymethamphetamine ('ecstasy') and 3,4-methylenedioxyethylamphetamine ('eve') isomers in rat brain and blood. *Neurosci. Lett.* 2002; **330**: 193.
19. Spitzer M, Franke B, Walter H, Buechler J, Wunderlich AP, Schwab M, Kovar KA, Hermle L, Gron G. Enantio-selective cognitive and brain activation effects of N-ethyl-3,4-methylenedioxyamphetamine in humans. *Neuropharmacology* 2001; **41**: 263.
20. Freudenmann RW, Spitzer M. The Neuropsychopharmacology and Toxicology of 3,4-methylenedioxy-N-ethyl-amphetamine (MDEA). *CNS. Drug Rev.* 2004; **10**: 89.
21. Mueller M, Peters FT, Maurer HH, McCann UD, Ricaurte GA. Non-linear pharmacokinetics of ({+/-})3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") and its major metabolites in squirrel monkeys at plasma concentrations of MDMA that develop after typical psychoactive doses. *J. Pharmacol. Exp. Ther.* 2008; **327**: 38.
22. Perfetti X, O'Mathuna B, Pizarro N, Cuyas E, Khymenets O, Almeida B, Pellegrini M, Pichini S, Monks TS, Lau SS, Farre M, Pascual JA, Joglar J, de la Torre R. Neurotoxic thioether adducts of MDMA identified in human urine after ecstasy ingestion. *Drug Metab. Dispos.* 2009;
23. Erives GV, Lau SS, Monks TJ. Accumulation of neurotoxic thioether metabolites of 3,4-(+/-)-methylenedioxymethamphetamine in rat brain. *J. Pharmacol. Exp. Ther.* 2008; **324**: 284.
24. Jones DC, Lau SS, Monks TJ. Thioether metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine inhibit human serotonin transporter (hSERT) function and simultaneously stimulate dopamine uptake into hSERT-expressing SK-N-MC cells. *J. Pharmacol. Exp. Ther.* 2004; **311**: 298.
25. Johnson MP, Nichols DE. Neurotoxic effects of the alpha-ethyl homologue of MDMA following subacute administration. *Pharmacol. Biochem. Behav.* 1989; **33**: 105.
26. Monks TJ, Jones DC, Bai F, Lau SS. The role of metabolism in 3,4-(+)-methylenedioxyamphetamine and 3,4-(+)-methylenedioxymethamphetamine (ecstasy) toxicity [review]. *Ther. Drug Monit.* 2004; **26**: 132.
27. Easton N, Marsden CA. Ecstasy: are animal data consistent between species and can they translate to humans? [review]. *J. Psychopharmacol.* 2006; **20**: 194.
28. Esteban B, O'Shea E, Camarero J, Sanchez V, Green AR, Colado MI. 3,4-Methylenedioxymethamphetamine induces monoamine release, but not toxicity, when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. *Psychopharmacology (Berl.)* 2001; **154**: 251.
29. Gollamudi R, Ali SF, Lipe G, Newport G, Webb P, Lopez M, Leakey JE, Kolta M, Slikker W. Influence of inducers and inhibitors on the metabolism in vitro and neurochemical effects in vivo of MDMA. *Neurotoxicology* 1989; **10**: 455.
30. Bai F, Lau SS, Monks TJ. Glutathione and N-acetylcysteine conjugates of alpha-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxyamphetamine-mediated neurotoxicity. *Chem. Res. Toxicol.* 1999; **12**: 1150.
31. Miller RT, Lau SS, Monks TJ. 2,5-Bis-(glutathion-S-yl)-alpha-methyldopamine, a putative metabolite of (+/-)-3,4-methylenedioxyamphetamine, decreases brain serotonin concentrations. *Eur. J. Pharmacol.* 1997; **323**: 173.
32. de la Torre R, Farre M. Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. *Trends Pharmacol. Sci.* 2004; **25**: 505.
33. Capela JP, Macedo C, Branco PS, Ferreira LM, Lobo AM, Fernandes E, Remiao F, Bastos ML, Dirnagl U, Meisel A, Carvalho F. Neurotoxicity mechanisms of thioether ecstasy metabolites. *Neuroscience* 2007; **146**: 1743.

34. McCann UD, Ridenour A, Shaham Y, Ricaurte GA. Serotonin neurotoxicity after (+/-)3,4- methylenedioxymethamphetamine (MDMA; "Ecstasy"): a controlled study in humans. *Neuropsychopharmacology* 1994; **10**: 129.
35. McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352**: 1433.
36. Crespi CL, Penman BW. Use of cDNA-expressed human cytochrome P450 enzymes to study potential drug-drug interactions. *Adv. Pharmacol.* 1997; **43**: 171.
37. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab. Rev.* 1997; **29**: 413.
38. Sesardic D, Boobis AR, Edwards RJ, Davies DS. A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the O-deethylation of phenacetin and is inducible by cigarette smoking. *Br. J. Clin. Pharmacol.* 1988; **26**: 363.
39. Distlerath LM, Reilly PE, Martin MV, Davis GG, Wilkinson GR, Guengerich FP. Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.* 1985; **260**: 9057.
40. Ortiz-de-Montellano PR, *Cytochrome P450 - Structure, Mechanism, and Biochemistry,* 3rd Kluwer Academic/Plenum Publishers: New York 2005;
41. Aktories K, Förstermann U, Hofmann F, Starke K, *Allgemeine und spezielle Pharmakologie und Toxikologie,* 9 Urban & Fischer: München 2004;
42. Crespi CL, Miller VP. The use of heterologously expressed drug metabolizing enzymes-state of the art and prospects for the future. *Pharmacol. Ther.* 1999; **84**: 121.
43. Salminen M, Lundstrom K, Tilgmann C, Savolainen R, Kalkkinen N, Ulmanen I. Molecular cloning and characterization of rat liver catechol-O-methyltransferase. *Gene* 1990; **93**: 241.
44. Lundstrom K, Salminen M, Jalanko A, Savolainen R, Ulmanen I. Cloning and characterization of human placental catechol-O-methyltransferase cDNA. *DNA Cell Biol.* 1991; **10**: 181.
45. Tilgmann C, Melen K, Lundstrom K, Jalanko A, Julkunen I, Kalkkinen N, Ulmanen I. Expression of recombinant soluble and membrane-bound catechol O-methyltransferase in eukaryotic cells and identification of the respective enzymes in rat brain. *Eur. J. Biochem.* 1992; **207**: 813.
46. Ulmanen I, Peranen J, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Bernasconi L, Aubry JP, Lundstrom K. Expression and intracellular localization of catechol O-methyltransferase in transfected mammalian cells. *Eur. J. Biochem.* 1997; **243**: 452.
47. Axelrod J, *Encyclopædia Britannica,* Encyclopædia Britannica Online: http://www.britannica.com 2009;
48. Axelrod J. Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. *Pharmacol. Rev.* 1966; **18**: 95.
49. Guldberg HC, Marsden CA. Catechol-O-methyl transferase: pharmacological aspects and physiological role. *Pharmacol. Rev.* 1975; **27**: 135.
50. Lautala P, Ulmanen I, Taskinen J. Molecular mechanisms controlling the rate and specificity of catechol O-methylation by human soluble catechol O-methyltransferase. *Mol. Pharmacol.* 2001; **59**: 393.
51. Thakker DR, Creveling CR. O-Methylation. In Mulder GJ (ed). *Conjugation Reactions in Drug Metabolism*, Taylor & Francis: London 1990; 193.
52. Zhu BT, Ezell EL, Liehr JG. Catechol-O-methyltransferase-catalyzed rapid O-methylation of mutagenic flavonoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity in vivo. *J. Biol. Chem.* 1994; **269**: 292.
53. Zhu BT, Liehr JG. Inhibition of catechol O-methyltransferase-catalyzed O-methylation of 2- and 4-hydroxyestradiol by quercetin. Possible role in estradiol-induced tumorigenesis. *J. Biol. Chem.* 1996; **271**: 1357.

54. Zhu BT, Patel UK, Cai MX, Conney AH. O-Methylation of tea polyphenols catalyzed by human placental cytosolic catechol-O-methyltransferase. *Drug Metab. Dispos.* 2000; **28**: 1024.
55. Zhu BT, Patel UK, Cai MX, Lee AJ, Conney AH. Rapid conversion of tea catechins to monomethylated products by rat liver cytosolic catechol-O-methyltransferase. *Xenobiotica* 2001; **31**: 879.
56. Zhu BT. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. *Curr. Drug Metab.* 2002; **3**: 321.
57. Vidgren J, Svensson LA, Liljas A. Crystal structure of catechol O-methyltransferase. *Nature* 1994; **368**: 354.
58. Vidgren J, Ovaska M. Structural aspects in the inhibitor design of catechol O-methyltransferase. In Veerapandian P (ed). *Structure-based Drug Design*, Marcel Dekker: New York 1997; 343.
59. Roth JA. Membrane-bound catechol-O-methyltransferase: a reevaluation of its role in the O-methylation of the catecholamine neurotransmitters. *Rev. Physiol. Biochem. Pharmacol.* 1992; **120**: 1.
60. Malherbe P, Bertocci B, Caspers P, Zurcher G, Da Prada M. Expression of functional membrane-bound and soluble catechol-O-methyltransferase in Escherichia coli and a mammalian cell line. *J. Neurochem.* 1992; **58**: 1782.
61. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 1995; **34**: 4202.
62. Lau EY, Bruice TC. Importance of Correlated Motions in Forming Highly Reactive Near Attack Conformations in Catechol O-Methyltransferase. *J. Am. Chem. Soc.* 1998; **120**: 12387.
63. Vidgren J, Ovaska M, Tenhunen J, Tilgmann C, Lotta T, Männistö PT. Catechol O-methyltransferase. In Cheng X, Blumenthal RM (eds). *Structure and Function of S-Adenosyl-L-Methionine-Dependent Methyltransferases*, World Scientific: Singapore 1999; 55.
64. Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol. Psychiatry* 2006; **60**: 141.
65. Lin LY, Di Stefano EW, Schmitz DA, Hsu L, Ellis SW, Lennard MS, Tucker GT, Cho AK. Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. *Drug Metab. Dispos.* 1997; **25**: 1059.
66. Kreth K, Kovar K, Schwab M, Zanger UM. Identification of the human cytochromes P450 involved in the oxidative metabolism of "Ecstasy"-related designer drugs. *Biochem. Pharmacol.* 2000; **59**: 1563.
67. Tucker GT, Lennard MS, Ellis SW, Woods HF, Cho AK, Lin LY, Hiratsuka A, Schmitz DA, Chu TY. The demethylenation of methylenedioxymethamphetamine ("ecstasy") by debrisoquine hydroxylase (CYP2D6). *Biochem. Pharmacol.* 1994; **47**: 1151.
68. Heydari A, Yeo KR, Lennard MS, Ellis SW, Tucker GT, Rostami-Hodjegan A. Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. *Drug Metab. Dispos.* 2004; **32**: 1213.
69. Meyer MR, Peters FT, Maurer HH. The Role of Human Hepatic Cytochrome P450 Isozymes in the Metabolism of Racemic MDMA and its Enantiomers. *Drug Metab. Dispos.* 2008; **36**: 2345.
70. Meyer MR, Peters FT, Maurer HH. The Role of Human Hepatic Cytochrome P450 Isozymes in the Metabolism of Racemic MDEA and its Single Enantiomers. *Drug Metab. Dispos.* 2009; **37**: 1152.
71. Meyer MR, Peters FT, Maurer HH. Stereoselective differences in the cytochrome P450-dependent dealkylation and demethylenation of *N*-methyl-benzodioxolyl-butanamine (MBDB, Eden) enantiomers. *Biochem. Pharmacol.* 2009; **77**: 1725.
72. Meyer MR, Peters FT, Maurer HH. Investigations on the Human Hepatic Cytochrome P450 Isozymes Involved in the Metabolism of 3,4-Methylenedioxy-amphetamine

(MDA) and Benzodioxolyl-butanamine (BDB) Enantiomers. *Toxicol. Lett.* 2009; in press

73. Meyer MR, Maurer HH. 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.* 2009; **22**: 1205.
74. Asanuma M, Miyazaki I, Ogawa N. Dopamine- or L-DOPA-induced neurotoxicity: the role of dopamine quinone formation and tyrosinase in a model of Parkinson's disease. *Neurotox. Res.* 2003; **5**: 165.

6 ABBREVIATIONS

MDMA	3,4-methylenedioxymethamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
MBDB	*N*-methyl-benzodioxolyl-butanamine
MDA	3,4-methylenedioxyamphetamine
BDB	benzodioxolyl-butanamine
CYP	cytochrome p450
COMT	catechol-O-methyltransferase
sCOMT	soluble form of catechol-O-methyltransferase
mbCOMT	membrane bound form of catechol-O-methyltransferase
HMEA	*N*-ethyl-4-hydroxy-3-methoxyamphetamine
DHMA	3,4-dihydroxymethamphetamine
DHEA	3,4-dihydroxyethylamphetamine
DHMBB	1,2-dihydroxy-4-[2-(methylamino)butyl]benzene
OR	oxidoreductase
cDNA	copy deoxyribonucleic acid
SAM	*S*-adenosylmethionine
HPLC	high pressure (performance) liquid chromatography
TEA	triethylamine
S-HFBPCl	S-heptafluoroproline chloride
MS	mass spectrometry
NICI	negative ion chemical ionization
SIM	selective ion monitoring
CNS	central nervous system
RSA	ring substituted amphetamines

GC	gas chromatography
SPE	solid phase extraction

7 ZUSAMMENFASSUNG

Im Rahmen dieser Dissertation wurde die Cytochrom P450 abhängige, enantioselektive *N*-Desalkylierung und Demethylenierung der Missbrauchsdrogen MDMA, MBDB und MDEA untersucht. Des Weiteren wurden Studien zur COMT-katalysierten *O*-Methylierung der catecholartigen Phase I Metabolite DHMA, DHEA und DHMBB sowie der Demethylenierung der *N*-Desalkyl-Metaboliten MDA und BDB angeschlossen. Die erhaltenen Daten dokumentieren eindeutig eine Präferenz für das *S*-Enantiomer der jeweiligen Stammverbindungen. Das Isoenzym CYP2C19 scheint in diesem Zusammenhang dasjenige Isoenzym mit der größten Enantioselektiviät zu sein. Die *N*-Desalkyl-Metaboliten der Ausgangsverbindungen wurden ebenfalls enantioselektiv, mit einer *S*-Präferenz, demethyleniert. Es war jedoch augenfällig, dass die Enantioselektivität bei diesen primären Aminen niedriger zu sein scheint als bei den sekundären Aminen. Auch die catecholartigen Phase I Metabolite der zuvor erwähnten Drogen unterliegen einer die *S*-Enantiomere bevorzugenden *O*-Methylierung. Diese Befunde können auch dazu beitragen, die *in vivo* beobachteten pharmakokinetischen Unterschiede der jeweiligen Enantiomere zu erklären. Abschließend wurden Hemmstudien mit den Metaboliten DHMA, DHEA und DHMBB durchgeführt. Diese zeigten eine nichtkompetetive Hemmung bezüglich der sCOMT-katalysierten 3-*O*-Methylierung von Dopamin. Diese Hemmung der physiologischen Methylierung könnte mit ein Grund für die beschriebene drogeninduzierte, irreversible Schädigung der Nervenzellendigungen sein.