Hepatic Cytochrome P450 Inhibition by Designer Drugs and Their Metabolites

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

von

Julia Dinger

Saarbrücken
2015

Tag des Kolloquiums: 12.02.2016

Dekan: Univ.-Prof. Dr.-Ing. Dirk Bähre

Berichterstatter: Univ.-Prof. Dr. rer. nat. Dr. h.c. Hans H. Maurer

Univ.-Prof. Dr. rer. nat. Rolf W. Hartmann

Vorsitz: Univ.-Prof. Dr. med. Veit Flockerzi

Akad. Mitarbeiter: Dr. rer. nat. Matthias Engel

Die folgende Arbeit entstand unter der Anleitung von Herrn Univ.-Prof. Dr. Dr. h.c. Hans H. Maurer in der Abteilung für Experimentelle und Klinische Toxikologie der Fachrichtung 2.4 Experimentelle und Klinische Pharmakologie und Toxikologie der Universität des Saarlandes in Homburg/Saar von Juni 2011 bis März 2015.

Mein besonderer Dank gilt:

Herrn Professor Hans H. Maurer für die herzliche Aufnahme in seinen Arbeitskreis, die Vergabe dieses spannenden, aber auch herausfordernden Dissertationsthemas, die Möglichkeit des eigenständigen Arbeitens, das Erlernen des analytischen Arbeitens, der aktiven Teilnahme an nationalen und internationalen Fachkongressen und seine ständige Diskussionsbereitschaft,

Herrn Professor Rolf W. Hartmann für die Übernahme des Koreferats,

Herrn Privatdozent Markus R. Meyer für seine unermüdliche Hilfe, wissenschaftliche Expertise und ständige Diskussionsbereitschaft, sowie die Weisheiten über Gott und die Welt, die mich stets zum Lachen brachten,

meinen Kolleginnen und Kollegen, für ihre Freundschaft und Hilfsbereitschaft während der Arbeits- und Dienstzeit, und die vielen schönen gemeinsam verbrachten Stunden, besonders meinen beiden Zimmernachbarn, die mich stets aufgemuntert und zum Lachen brachten,

Frau Jessica Welter-Lüdecke für die im Studium gereifte und trotz großer Entfernung immer noch bestehende Freundschaft und Unterstützung während der ganzen Zeit,

Herrn Armin Weber für seine Gelassenheit, Ruhe, die Stunden vor der Q-Exactive, sowie seinen Rat bei chromatographischen oder massenspektrometrischen Problemen,

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

meinen Eltern und meiner Oma, die mich in meinem Tun immer unterstützt haben, in schwierigen Zeiten aufgeheitert haben und immer an mich geglaubt haben,

Patrick und Lars für die einzigartige Freundschaft und dass sie mich immer zum Lachen gebracht haben, auch wenn es mir nicht zum Lachen zumute war.

Für die, die immer an mich geglaubt haben

Monde und Jahre vergehen, aber ein schöner Moment leuchtet das Leben hindurch

Franz Grillparzer

TABLE OF CONTENTS

1 GENERAL PART

1.1 Introduction

1.1.1 Human Cytochrome P450 (CYP) Isoenzymes

The human cytochrome P450 (CYP) isoenzymes are members of the superfamily of hemeproteins. They are components of the mixed-function oxidase systems and essential protein catalysts for the oxidative metabolism of many xeno- and endobiotics [1]. Two classes of CYPs are defined. The class I is located in bacteria and mitochondria. The human CYPs belong to class II, also known as microsomal CYPs [1]. To date 57 different human CYPs have been identified and 15 of these isoenzymes are involved in the metabolism of xenobiotics. They are mainly located in the endoplasmic reticulum of liver cells. As depicted in Figure 1, CYP isoenzymes are responsible for about 75 % of the metabolism of xenobiotics [2]. Within these isoenzymes, nearly 100 % of the reactions are prepared by seven isoenzymes, namely CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. However the largest fraction of the CYP reactions was catalyzed by CYP3A isoenzymes [3]. Within these xenobiotic-metabolizing isoenzymes, CYP2C9, CYP2C19, and CYP2D6 were highly polymorphic expressed leading to different CYP activities. Poor metabolizers (PM) showed a complete lack of certain isoenzyme activity, intermediate metabolizers (IM) a decreased activity, the extensive metabolizers (EM) a normal activity, and the ultra-rapid metabolizers an increased activity [4]. As consequence, different plasma levels of the drugs and/or their metabolites can result varying from too low to too high. As shown in the study of Stamer et al., the same effect could be achieved by inhibition of the metabolizing enzyme [5], showing the importance of drug-drug interaction studies.

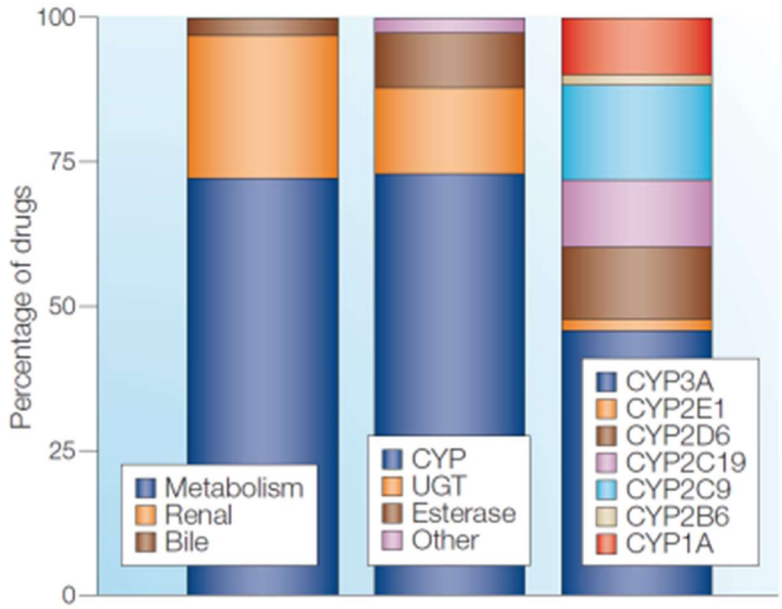


Figure 1: Elimination routes of drugs, adapted from Wienkers and Heath [2]

1.1.2 Drug-Drug-Interactions

Dug-drug interactions (DDI) were known as situation in which one drug influence the effect and/or the pharmacokinetics of co-administered drugs. These interactions could be caused e.g. by CYP inhibition or induction. Thereby, induction is defined as increase of enzyme amount as consequence of long-term drug exposure. In contrast, the inhibition of a CYP enzyme results in a decrease of the enzyme activity and thus, in a decreased metabolism rate of a drug by co-administered drug [6]. More and more patients are nowadays treated with more than one drug at the same time increasing the risk of DDI, which may lead to more frequent and longer hospitalization [7]. Population-based studies between the early 1990's and 2000's showed that the amount of polypharmacy (prescribing of more than 5 drugs) and the associated DDI increased, illustrating the huge problem with DDI [8-11]. One popular example for such an interaction was described by Gasche et al. [12]. A patient under treatment with therapeutic doses of codeine and clarithromycin showed a narcotic syndrome. Clarithromycin inhibited CYP3A4, which was responsible for the demethylation of codeine to the inactive norcodeine. This led to an

increased metabolism rate to morphine by CYP2D6 and the patient was a CYP2D6 ultra rapid metabolizer, which enhanced the morphine formation again. Furthermore he also was kidney insufficiency, leading to a reduction of the excretion of the active morphin-6-glucuronide. All these circumstances contributed the narcotic syndrome, but the CYP3A4 inhibition was a main part. However, DDI were not only between therapeutic drugs, but also between drugs, drugs of abuse, and food ingredients. Grapefruit juice is one important example [13]. The ingredients of this citrus fruit were able to inhibit CYP3A4 activity irreversible. Because the risk of adverse side effects by CYP inhibition is well known and documented, new therapeutic drugs have to be screened before approval. In contrast, new drugs of abuse were marketed and consumed without such testing. However, various studies have shown that drugs of abuse were CYP inhibitors comparable to known clinically relevant inhibitors [14-16].

1.1.3 In vitro CYP Inhibition Studies

1.1.3.1 In Vitro Enzyme Kinetic Studies

The knowledge about the Michaelis Menten constant (K_m) of a respective biotransformation reaction is essential for the determination of the CYP inhibition using the inhibition constant 50 value (IC_{50}) [17]. According to the U.S. Food and Drug Administration (FDA) guidance, the used model substrate concentration to determine the inhibition potential of a new substance should be at or below K_m [17]. As a wide range of K_m values are known for such substrates, the K_m value should be evaluated for the used incubation system to guarantee the use of optimal substrate amount. The K_m value is defined as the substrate concentration that will yield a reaction velocity that is half of the maximal velocity (V_{max}) and reflects the substrate affinity to a certain enzyme. Conventional determinations of enzyme kinetic parameters are made by assessing the rate of product (metabolite) formation at several substrate concentrations [18]. The simplest model to describe enzymatic biotransformation and hence to calculate K_m is fitting the initial rate velocities at various substrate concentrations to the Michaelis Menten equation (eq. 1).

$$V_{enzyme} = V_{max} \times [S] / (K_m + [S]) \quad (1)$$

For ideal determinations, the protein concentrations and incubation time should be within the linear range of metabolite formation, and in total, less than 20% of substrate should be consumed.

1.1.3.2 In Vitro CYP Inhibition Assays

For determination the in vitro inhibition potential of a substance, there were two assay opportunities: a single assay approach, monitoring every single isoenzyme activity separately or a cocktail approach, which is able to determine the inhibition potential against many isoenzymes at the same time [19-25]. For monitoring the isoenzyme inhibition, specific substrates are needed. Such a substance should show two characteristics, single CYP selectivity and simple metabolic scheme. These substrates were well studied and listed in a guidance of the FDA [7]. The second opportunity is time and cost saving, because fewer amounts of isoenzymes and cofactors are needed. Time efficiency can also be achieved because all interested isoenzymes could be monitored at the same time. However, the cocktail approach could have some limitations due to interferences between the specific substrates and metabolites, which have to be tested in advance. The interaction potential of bupropion, the CYP2B6 test substrate, with CYP2D6 was well documented [14]. Pooled human liver microsomes (pHLM) containing all relevant CYP isoenzymes were favored as isoenzyme source. The IC_{50} value is mainly used as constant to describe the inhibition potential [19-25]. The IC_{50} value represents the inhibitor concentration, at which the enzyme activity is reduced by 50 %. For that purpose, the metabolite concentration formed by the corresponding CYPs had to be determined in absence or presence of different inhibitor concentrations. Therefore, a multi-analyte approach is needed that allows quantifying low concentrations of the metabolites of all relevant CYP model substrates in the incubation mixtures besides the model substrates and inhibitors. For that application, LC-MS techniques were preferable used in lots of published assays [19-25]. The high sensitivity and selectivity of those techniques allowed quantification of metabolites especially if the turn-over rate was reduced by strong inhibition. Besides the test substrates, test inhibitors were also well studied [17]. These inhibitors were necessary for the evaluation of a new cocktail assay. The determined IC_{50} values of the cocktail assay could be confirmed by

comparing the values determined with single approach and with those given in the literature. These test inhibitors could also be used as positive control during isoenzyme incubations as described in part 2.2 [26].

1.1.4 In Vivo CYP Inhibition Studies

In vivo studies were be performed if the in vitro data give hints for a CYP inhibition by the corresponding drug. The studies were generally designed to compare substrate concentrations with and without the in vitro tested inhibitor [27]. For the study design, crossover or parallel design is advised by the FDA. The crossover design is more efficient, but more time-consuming than the parallel design. Concerning the doses, the substrate and the inhibitor should be administered at therapeutic range. Another important part of the study design is the application way of the substrate and the inhibitor. To monitor a rapid reversible inhibition, the substrate and the inhibitor should be given simultaneously. To monitor a mechanism-based inhibition, the inhibitor should be given prior to the substrate. Beside the right study design, the choice of the right test substrates is more important for in vivo studies than for in vitro studies. They should show the same characteristics as the in vitro test substrates. In addition their pharmacologic profile should be as harmless as possible. For in vivo CYP inhibition testing, the substrates could also be used in single assay or cocktail assay, as described in the literature [28, 29]. The advantages of the cocktail assay were the same as already described under 1.1.3.2 for the in vitro cocktail assays. For the in vivo testing of CYP1A2, caffeine is a suitable test substrate. It is exclusively metabolized by CYP1A2 in humans and rats and only the metabolite profile differs between these species [30].However, these differences did not influence the explanatory power by monitoring the pharmacokinetic parameters of caffeine. To give a statement about the inhibition potential of the tested drug, the area under the plasma concentration/time curve (AUC) of the test substrate could be used. The AUC could be calculated using the simple trapezoidal rule [31]. Reduced metabolism by inhibition leads to increased AUC. According to this increase the strength of the inhibitors can be classified. Strong inhibitors increased the AUC more than five times, moderate inhibitors more than two times, and weak inhibitors more

than one time. For CYP1A2, fluvoxamine is known as strong inhibitor [27] and can be used as test inhibitor to proof the study design.

1.1.5 Drugs of Abuse

In the last few years, the drugs of abuse (DOA) market was rapidly growing around the world [32, 33]. The early warning system of the European Monitoring Centre of Drugs and Drug Addiction (EMCDDA) reported 81 new psychoactive substances in 2013 in contrast to 15 in 2005. Most of the new substances were produced to get legal alternatives for controlled drugs [34], purchased via internet as so-called “legal highs” or as research chemicals. Most of these drugs belong to the groups of phenethylamines, piperazines, synthetic cannabinoids, or tryptamines [32]. A huge health risk arises from the fact that these substances are purchased and consumed without any safety testing. Therefore, no or only few data are available about the pharmacodynamic, -kinetic or the toxicological effects of these new substances.

1.1.5.1 Methylendioxy-derived designer drugs

The class of methylenedioxy-derived designer drugs (MDD) includes various families of compounds as given in Figure 2. Beside the classic amphetamine derivatives such as methylenedioxy-methamphetamine (MDMA), methylenedioxy derivatives of cathinones, piperazines, pyrrovalerones, and tryptamines appeared in the last few years. With the exception of the tryptamines, all derivatives act in the central nervous system (CNS) as stimulants affecting directly or indirectly the release or persistence of monoamine neurotransmitters such as serotonin (5-HT), noradrenalin (NA) and dopamine (DA) from presynaptic nerves [35]. In contrast, the tryptamines were 5-HT_{1A} and 5-HT_{2A} receptor agonists resulting in hallucinogenic effects [36]. According to the World and European Drug Report, the older methylenedioxy stimulants such as methylenedioxy-amphetamine (MDA), MDMA or methylenedioxy-ethylamphetamine (MDEA) were still in use [32, 33]. However only for MDMA, data about the CYP inhibition potential were available so far [37, 38]. But

in recent years, series of novel MDD came onto the DOA market [32]. Cathinones such as methylone and MDPV were sold as so-called “bath salts” via the internet and caused fatal poisonings [39-41]. To date, the knowledge about the interaction potential caused by CYP inhibition is not known for most of these compounds. Only a study about methylone could show that this compound also inhibited CYP2D6 activity as its amphetamine analogue MDMA [15]. Recently, benzofuranes could be detected in drug and human body samples as alternatives for MDMA [42-44]. They were not classic methylenedioxy-derived drugs, but as bioisosteric analogues, they can be assigned to this group [43]. The second oxygen of the methylenedioxy ring is substituted by one methine group, resulting in comparable effects of the CNS. Information about the pharmacological effects on neurotransmission was already known [43]. For all these described substances with the exception of MDMA and methylone, no data were available about the inhibition potential of the different CYP isoenzymes.

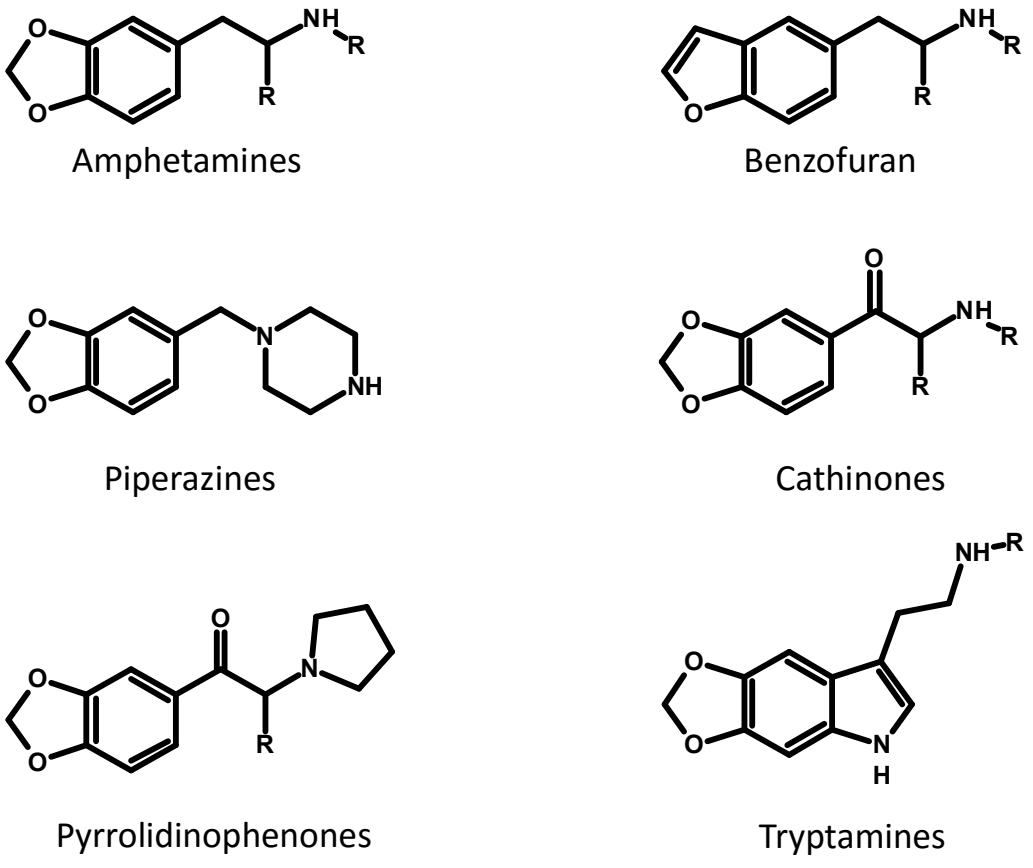
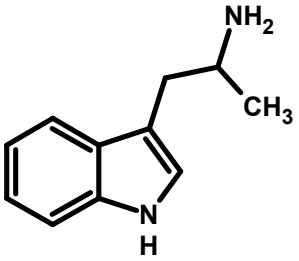


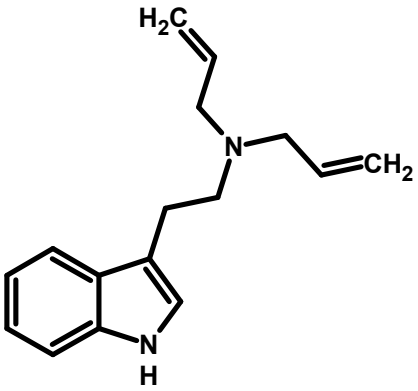
Figure 2: Structures of methylenedioxy-derived designer drug classes

1.1.5.2 Tryptamine-derived designer drugs

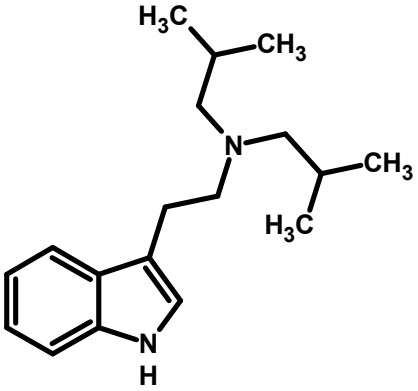
Biogenic tryptamines such as psilocybin and *N,N*-dimethyltryptamine (DMT) have been used since ancient times by South Americans for their hallucinogenic effects [36]. They act as agonist of the 5-HT_{2A} receptor, resulting in mood, sensory perception, and thought changes. Today there were used as alternatives for traditional hallucinogens [45]. These substances as well as other hallucinogens like LSD were under legislative control, so users were searching for new, unscheduled alternatives. The market for newer tryptamines was opened. Alexander and Ann Shulgin synthesized hundreds of these substances and about 50 of them were psychoactive. They described the syntheses, doses and adverse side effects in their book “THIKAL” [46]. Several tryptamine-derived new psychoactive substances (TDNPS) such as alpha-methyl tryptamines (AMT), dimethyl tryptamines (DMT), diallyl tryptamines (DALT), and diisopropyl tryptamines (DiPT, structures given in Figure 3) entered the drugs of abuse market over the last few years. They were classified as one of the drug classes of the so-called New Psychoactive Substances (NPS). The recently published United Nations Office on Drugs and Crime World Drug Report [33] indicated that up to July 2012, twenty-five new tryptamines were reported by member states [33]. As mentioned above, most of them are not yet under legislative control and thus consumed as so-called “legal highs” purchased mainly via internet. A study evaluating legal high products available in the UK showed that 13 % of them contained hallucinogenic tryptamines [47]. Intoxication cases with 5-methoxy-*N,N*-diisopropyl tryptamine (5-MeO-DiPT) were reported since the 2000’s [48-52]. The newer DALT analogue, 5-methoxy-*N,N*-diallyltryptamine (5-MeO-DALT), caused fatal poisonings [53, 54]. Both, the reports about intoxications and the availability via internet, showed the distribution of these NPS. Again, they were also purchased and consumed without any pharmacologic safety testing.



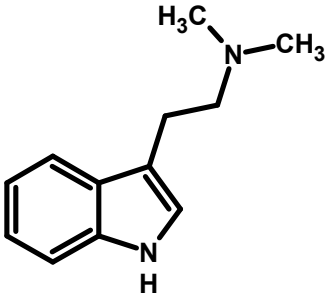
AMT



DALT



DiPT



DMT

Figure 3: Structures of some tryptamine-derived NPS

1.2 Aimes and Scopes

As mentioned above, CYP isoenzymes are responsible for most of the metabolism of xenobiotics and endogenous molecules [1]. In vitro CYP inhibition cocktail assays are common approaches to test the inhibition potential of new drugs. New therapeutic drug candidates have to be tested before approval and corresponding data can be found e.g. in the Internet (http://medicine.iupui.edu/clinpharm/ddis/main-table/). In contrast, inhibition data of most new psychoactive substances are not available because such drugs are marketed and consumed without any safety pharmacology testing.

Therefore, the aims of the presented studies were:

- Development and validation of an LC-HR-MS/MS method to quantify low concentrations of the different CYP model metabolites in presence of the model substrates and inhibitors
- Development and validation of an in vitro all-in-one CYP inhibition approach (cocktail approach) for assessing the inhibition potential of drugs of abuse
- Determination of the CYP inhibition potential (IC_{50} value) of methylenedioxy-derived designer drugs of different drug classes
- Determination of the CYP inhibition potential (IC_{50} value) of new psychoactive substances of the tryptamine class
- Determination of the in vivo CYP1A2 inhibition potential of 5-MeO-DALT

2 PUBLICATIONS OF THE RESULTS

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

2.1 Development and validation of a liquid-chromatography high-resolution tandem mass spectrometry approach for quantification of nine cytochrome P450 (CYP) model substrate metabolites in an in vitro CYP inhibition cocktail [55]

(DOI: 10.1001/S00216-014-7849-X)

2.2 Development of an in vitro cytochrome P450 cocktail inhibition assay for assessing the inhibition risk of drugs of abuse [26] (DOI: 10.1016/J.TOXLET.2014.08.004)

2.3 In vitro cytochrome P450 inhibition potential of methylenedioxy-derived designer drugs studied with a two cocktail approach [56] (DOI: 10.1007/s00204-014-1412-6)

2.4 Cytochrome P450 inhibition potential of new psychoactive substances of the tryptamine class [57]

(DOI 10.1016/j.toxlet.2015.11.013)

3 FINAL DISCUSSION AND CONCLUSIONS

The presented studies showed that methylenedioxy-derived designer drugs as well as tryptamine-derived novel psychoactive substances were inhibitors of several CYP isoenzymes. The developed and validated LC-HR-MS/MS approach was sensitive enough for the quantification of low substrate metabolite concentrations. Although some validation results were outside the acceptance criteria for one-point or/and full calibration, the method could be used for determination of IC_{50} values of the drugs of abuse. For *O*-deethyl phenacetin, 5-hydroxy omeprazole, *O*-demethyl dextromethorphan, and 6-hydroxy chlorzoxazone, one-point calibration could be used for quantification without any problem. For the other analytes, one-point calibration could also be used, although some results were outside the acceptance criteria. Full calibration did not provide better results, but the calculation of IC_{50} values with the exception of *N*-deethyl amodiaquine was possible. However, a general statement of CYP 2C8 inhibition was also possible [55]. During the assay development, some interference between the substrates and metabolites could be detected. As consequence, the cocktail assay had to be split. However, the pooled mixture of both incubates could be analyzed in one analytical run. The new inhibition cocktail assay was comparable with single assay results, reproducible, and applicable for testing the inhibition potential of drugs of abuse [26]. All of the tested methylenedioxy-derived designer drugs inhibited CYP2D6 activity. In addition correlations between the IC_{50} value and the structural properties could be seen. Therefore, it seemed that the inhibition potential of substances with unsubstituted amine function decrease compared to those with lipophilic substituent. This could be seen for the amphetamine types as well as for the cathinones. A second correlation was that the IC_{50} values decreased from drugs with methyl to those with ethyl side chains in alpha position. For the 2,3-methylenedioxy compounds the values were smaller than that for the 3,4-substituted analogues. Concerning the benzofurans, bioisosteric derivatives of 3,4-MDA and 3,4-MDMA, the IC_{50}

values decreased from the amphetamines to the benzofurans. The additional beta-keto function of the cathinones led to an increased inhibition against CYP2D6 in comparison to the amphetamines. This beta-keto group was also responsible for the CYP2B6 inhibition in contrast to the amphetamines, which showed no inhibition on that isoenzyme. Further six drugs showed inhibition of the CYP1A2 activity, three of CYP2A6 activity, 13 of CYP2B6 activity, two of CYP2C9 activity, six of CYP2C19 activity, one of CYP2E1 activity, and six of CYP3A activity [56]. Concerning the tryptamines, all tested TDNPS with the exception of DMT showed more or less inhibition potential against the various CYP isoforms. The following drugs showed inhibition of the activity of the given CYPs, which might be clinically relevant: CYP1A2, 4-MeO-AMT, 5-F-2-Me-AMT, 5-Cl-AMT, 6-F-AMT, 7-Me-AMT, all DALTs; CYP2A6, AMT, 6-F-AMT; CYP2D6, AMT, 5-F-AMT, 5-F-2-Me-AMT, 5-Cl-AMT, all DALTs, 4-HO-DiPT; CYP2E1, 5-Me-DALT, 5-MeO-DALT, 5-MeO-2-Me-DALT; CYP3A, 5-F-2-Me-DALT, 5-Cl-DALT, 5-Br-DALT, 5-Me-DALT, 6-F-DALT [57]. In addition to this in vitro data of the TDNPS, the inhibition potential of 5-MeO-DALT could be confirmed by an in vivo experiment using rats and caffeine as probe substrate. The kinetic parameters of caffeine changed with the coadministration of 5-MeO-DALT as expected from the in vitro results. Because of the inhibition potential in the range of clinical relevant inhibitors and plasma concentrations similar to them, CYP inhibition by MDD and TDNPS might be clinically relevant [56]. Thus, they could change the pharmacokinetics, namely the plasma levels and the corresponding effects of co-administered therapeutics or NPS. Prodrugs such as tramadol, mainly metabolized by one isoenzyme, could lose their potency, if bioactivation was blocked [5]. However, it should be kept in mind that clinical cases or studies would be desirable to assess the risks associated with drug-drug interactions.

4 REFERENCES

1. Ortiz-de-Montellano PR (2005) Cytochrome P450 - Structure, Mechanism, and Biochemistry. Kluwer Academic/Plenum Publishers, New York
2. Wienkers LC, Heath TG (2005) Predicting in vivo drug interactions from in vitro drug discovery data. Nat Rev Drug Discov 4:825-833
3. Rendic SP, Guengerich FP (2014) Survey of Human Oxidoreductases and Cytochrome P450 Enzymes Involved in the Metabolism of Chemicals. Chem Res Toxicol -DOI: 10.1021/tx500444e
4. Johansson I, Ingelman-Sundberg M (2011) Genetic polymorphism and toxicology--with emphasis on cytochrome p450. Toxicol Sci 120:1-13
5. Stamer UM, Musshoff F, Kobilay M, Madea B, Hoeft A, Stuber F (2007) Concentrations of Tramadol and O-desmethyltramadol Enantiomers in Different CYP2D6 Genotypes. Clin Pharmacol Ther 82:41-47
6. Pelkonen O, Maenpaa J, Taavitsainen P, Rautio A, Raunio H (1998) Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiotica 28:1203-1253
7. Palleria C, Di PA, Giofre C, Caglioti C, Leuzzi G, Siniscalchi A, De SG, Gallelli L (2013) Pharmacokinetic drug-drug interaction and their implication in clinical management. J Res Med Sci 18:601-610
8. Guthrie B, Makubate B, Hernandez-Santiago V, Dreischulte T (2015) The rising tide of polypharmacy and drug-drug interactions: population database analysis 1995-2010. BMC Med 13:74

9. Haider SI, Johnell K, Thorslund M, Fastbom J (2007) Trends in polypharmacy and potential drug-drug interactions across educational groups in elderly patients in Sweden for the period 1. Int J Clin Pharmacol Ther 45:643-653
10. Nobili A, Pasina L, Tettamanti M, Lucca U, Riva E, Marzona I, Monesi L, Cucchiani R, Bortolotti A, Fortino I, Merlino L, Walter LG, Giuliani G (2009) Potentially severe drug interactions in elderly outpatients: results of an observational study of an administrative prescription database. J Clin Pharm Ther 34:377-386
11. Franchi C, Tettamanti M, Pasina L, Djignefa CD, Fortino I, Bortolotti A, Merlino L, Nobili A (2014) Changes in drug prescribing to Italian community-dwelling elderly people: the EPIFARM-Elderly Project 2000-2010. Eur J Clin Pharmacol 70:437-443
12. Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P, Desmeules J (2004) Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N Engl J Med 351:2827-2831
13. Bailey DG, Dresser G, Arnold JM (2013) Grapefruit-medication interactions: forbidden fruit or avoidable consequences? CMAJ 185:309-316
14. Ewald AH, Maurer HH (2008) 2,5-Dimethoxyamphetamine-derived designer drugs: Studies on the identification of cytochrome P450 (CYP) isoenzymes involved in formation of their main metabolites and on their capability to inhibit CYP2D6. Toxicol Lett 183:52-57
15. Pedersen AJ, Petersen TH, Linnet K (2013) In vitro metabolism and pharmacokinetic studies on methylone. Drug Metab Dispos 41:1247-1255

16. Wu D, Otton SV, Inaba T, Kalow W, Sellers EM (1997) Interactions of amphetamine analogs with human liver CYP2D6. Biochem Pharmacol 53:1605-1612

17. U.S.Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER) (2006) Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling [Draft]

18. Obach RS, 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

19. Ventura V, Sola J, Peraire C, Bree F, Obach R (2012) In vitro evaluation of the interaction potential of irosustat with drug-metabolizing enzymes. Drug Metab Dispos 40:1268-1278

20. Dierks EA, Stams KR, Lim HK, Cornelius G, Zhang H, Ball SE (2001) A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using an in vitro cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry. Drug Metab Dispos 29:23-29

21. Kim MJ, Kim H, Cha IJ, Park JS, Shon JH, Liu KH, Shin JG (2005) High-throughput screening of inhibitory potential of nine cytochrome P450 enzymes in vitro using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 19:2651-2658

22. Turpeinen M, Uusitalo J, Jalonen J, Pelkonen O (2005) Multiple P450 substrates in a single run: rapid and comprehensive in vitro interaction assay. Eur J Pharm Sci 24:123-132
23. Lee KS, Kim SK (2013) Direct and metabolism-dependent cytochrome P450 inhibition assays for evaluating drug-drug interactions. J Appl Toxicol 33:100-108
24. Qin CZ, Ren X, Tan ZR, Chen Y, Yin JY, Yu J, Qu J, Zhou HH, Liu ZQ (2014) A high-throughput inhibition screening of major human cytochrome P450 enzymes using an in vitro cocktail and liquid chromatography-tandem mass spectrometry. Biomed Chromatogr 28:197-203
25. Qiao X, Ji S, Yu SW, Lin XH, Jin HW, Duan YK, Zhang LR, Guo DA, Ye M (2014) Identification of Key Licorice Constituents Which Interact with Cytochrome P450: Evaluation by LC/MS/MS Cocktail Assay and Metabolic Profiling. AAPS J 16:101-113
26. Dinger J, Meyer MR, Maurer HH (2014) Development of an in vitro cytochrome P450 cocktail inhibition assay for assessing the inhibition risk of drugs of abuse. Toxicol Lett 230:28-35
27. U.S Deparment of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) (2012) Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations [Draft]
28. Bosilkovska M, Samer CF, Deglon J, Rebsamen M, Staub C, Dayer P, Walder B, Desmeules JA, Daali Y (2014) Geneva cocktail for cytochrome p450 and P-glycoprotein activity assessment using dried blood spots. Clin Pharmacol Ther 96:349-359

29. Donzelli M, Derungs A, Serratore MG, Noppen C, Nezic L, Krahenbuhl S, Haschke M (2014) The basel cocktail for simultaneous phenotyping of human cytochrome P450 isoforms in plasma, saliva and dried blood spots. Clin Pharmacokinet 53:271-282
30. Kot M, Daniel WA (2008) Caffeine as a marker substrate for testing cytochrome P450 activity in human and rat. Pharmacol Rep 60:789-797
31. Chiou WL (1978) Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level--time curve. J Pharmacokinet Biopharm 6:539-546
32. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2014) European Drug Report: Trends and developments. http://www emcdda europa eu/attachements cfm/att_228272_EN_TDAT14001ENN pdf
33. United Nations Office on Drugs and Crime (UNODC) (2014) World Drug Report 2014. http://www.unodc.org/documents/data-and-analysis/WDR2014/World_Drug_Report_2014_web.pdf
34. Musselman ME, Hampton JP (2014) "Not for human consumption": a review of emerging designer drugs. Pharmacotherapy 34:745-757
35. Iversen L, White M, Treble R (2014) Designer psychostimulants: pharmacology and differences. Neuropharmacology 87:59-65
36. Araujo AM, Carvalho F, Bastos ML, Guedes de PP, Carvalho M (2015) The hallucinogenic world of tryptamines: an updated review. Arch Toxicol - DOI:10.1007/s00204-015-1513-x
37. Heydari A, Yeo KR, Lennard MS, Ellis SW, Tucker GT, Rostami-Hodjegan A (2004) Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. Drug Metab Dispos 32:1213-1217

38. O'Mathuna B, Farre M, Rostami-Hodjegan A, Yang J, Cuyas E, Torrens M, Pardo R, Abanades S, Maluf S, Tucker GT, de la TR (2008) The consequences of 3,4-methylenedioxymethamphetamine induced CYP2D6 inhibition in humans. J Clin Psychopharmacol 28:523-529

39. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2014) Report on the risk assessment of 1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one (MDPV) in the framework of the Council Decision on new psychoactive substances. http://www emcdda europa eu/attachements cfm/att_228256_EN_MDPV%20RAR_with%20annexes pdf

40. Coppola M, Mondola R (2012) 3,4-methylenedioxypyrovalerone (MDPV): chemistry, pharmacology and toxicology of a new designer drug of abuse marketed online. Toxicol Lett 208:12-15

41. Pearson JM, Hargraves TL, Hair LS, Massucci CJ, Frazee CC, III, Garg U, Pietak BR (2012) Three fatal intoxications due to methylone. J Anal Toxicol 36:444-451

42. Advisory Council on the Misuse of Drugs (ACMD) (2013) Benzofurans: A review of the evidence of use and harm. https://www gov uk/government/uploads/system/uploads/attachment_data/file/261783/Benzofuran_compounds_report pdf

43. Welter J, Kavanagh P, Meyer MR, Maurer HH (2015) Benzofuran analogues of amphetamine and methamphetamine: studies on the metabolism and toxicological analysis of 5-APB and 5-MAPB in urine and plasma using GC-MS and LC-(HR)-MS^n techniques. Anal Bioanal Chem 407:1371-1388

44. Welter J, Brandt SD, Kavanagh P, Meyer MR, Maurer HH (2015) Metabolic fate, mass spectral fragmentation, detectability, and differentiation in urine of the

benzofuran designer drugs 6-APB and 6-MAPB in comparison to their 5-isomers using GC-MS and LC-(HR)-MS techniques. Anal Bioanal Chem 407:3457-3470

45. Winstock AR, Kaar S, Borschmann R (2014) Dimethyltryptamine (DMT): prevalence, user characteristics and abuse liability in a large global sample. J Psychopharmacol 28:49-54
46. Shulgin ATShulgin A (1997) Tihkal, The Continuation. Transform Press, Berkley (CA)
47. Schmidt MM, Sharma A, Schifano F, Feinmann C (2011) "Legal highs" on the net-Evaluation of UK-based Websites, products and product information. Forensic Sci Int 206:92-97
48. Meatherall R, Sharma P (2003) Foxy, a designer tryptamine hallucinogen. J Anal Toxicol 27:313-317
49. Vorce SP, Sklerov JH (2004) A general screening and confirmation approach to the analysis of designer tryptamines and phenethylamines in blood and urine using GC-EI-MS and HPLC-electrospray-MS. J Anal Toxicol 28:407-410
50. Wilson JM, McGeorge F, Smolinske S, Meatherall R (2005) A foxy intoxication. Forensic Sci Int 148:31-36
51. Tanaka E, Kamata T, Katagi M, Tsuchihashi H, Honda K (2006) A fatal poisoning with 5-methoxy-N,N-diisopropyltryptamine, Foxy. Forensic Sci Int 163:152-154
52. Smolinske SC, Rastogi R, Schenkel S (2005) Foxy methoxy: a new drug of abuse. J Med Toxicol 1:22-25

53. Corkery JM, Durkin E, Elliott S, Schifano F, Ghodse AH (2012) The recreational tryptamine 5-MeO-DALT (N,N-diallyl-5-methoxytryptamine): a brief review. Prog Neuropsychopharmacol Biol Psychiatry 39:259-262

54. Elliott S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243:55-60

55. Dinger J, Meyer MR, Maurer HH (2014) Development and validation of a liquid-chromatography high-resolution tandem mass spectrometry approach for quantification of nine cytochrome P450 (CYP) model substrate metabolites in an in vitro CYP inhibition cocktail. Anal Bioanal Chem 406:4453-4464

56. Dinger J, Meyer MR, Maurer HH (2014) In vitro cytochrome P450 inhibition potential of methylenedioxy-derived designer drugs studied with a two cocktail approach. Arch Toxicol -DOI: 10.1007/s00204-014-1412-6

57. Dinger J, Woods C, Brandt SD, Meyer MR, Maurer HH (2016) Cytochrome P450 inhibition potential of new psychoactive substances of the tryptamine class. Toxicol Lett 241:82-94

5 ABBREVIATIONS

AMT	Alpha-methyl tryptamine
AUC	Area under the plasma concentration/ time curve
CNS	Central nervous system
CYP	Cytochrome P450
DA	Dopamine
DALT	Diallyl tryptamine
DDI	Drug-drug-interactions
DiPT	Diisopropyl tryptamine
DMT	*N,N*-Dimethyltryptamine
DOA	Drugs of abuse
EMCDDA	European Monitoring Centre of Drugs and Drug Addiction
5-HT	Serotonin
IC_{50}	Inhibition constant 50
K_m	Michaelis Menten constant
LC	Liquid chromatography
MDA	methylenedioxy-amphetamine
MDD	Methylenedioxy-derived designer drugs
MDEA	methylenedioxy-ethylamphetamine
MDMA	methylenedioxy-methamphetamine
5-MeO-DALT	5-methoxy-*N,N*-diallyl tryptamine
5-MeO-DiPT	5-methoxy-*N,N*-diisopropyl tryptamine
MS	Mass spectrometry
NA	Noradrenaline
NPS	New psychoactive substances
TDNPS	tryptamine-derived new psychoactive substances
V_{max}	Maximal velocity

6 SUMMARY

In the presented studies, a CYP inhibition cocktail assay was developed and validated. The LC-HR-MS/MS method was able to quantify low concentrations of the metabolites using a one-point calibration model. During development, the cocktail assay showed interferences between the substrates and metabolites. It was spilt into cocktail A consisting of phenacetin, coumarin, diclofenac, dextromethorphan, and testosterone and cocktail B of bupropion, amodiaquine, omeprazole, and chlorzoxazone. Using these sets, the IC_{50} values of specific test inhibitors were comparable with the values determined with a single approach and with those given in the literature. Altogether, the new assay provided valid, reproducible, and applicable results. All the tested drugs of abuse showed inhibition against at least one isoenzyme. The methylenedioxy-derived designer drugs inhibited CYP2D6 activity with values comparable to paroxetine or fluoxetine. The methylendioxy-ethylphenethylamines showed the highest potential against CYP2D6. The cathinones showed also inhibition on the CYP2B6 activity comparable to efavirenz. Overall, CYP1A2, CYP2A6, 2B6, 2D6, and CYP3A4 were inhibited similar to known inhibitors. The tested DALTs showed the strongest inhibition against CYP1A2, CYP2D6, and CYP3A activity similar to known inhibitors. For 5-MeO-DALT, CYP1A2 inhibition could also be shown during an in vivo experiment with rats as expected from the in vitro results.

7 ZUSAMMENFASSUNG

Diese Dissertation beschreibt die Entwicklung und Validierung eines CYP Inhibitions-Cocktail-Assay. Die LC-HR-MS/MS Methode erlaubte niedrige Konzentrationen an Metaboliten mittels Ein-Punkt-Kalibration zu bestimmen. Während der Entwicklung zeigte der Cocktail-Assay Interferenzen zwischen den Substraten und den Metaboliten. Daher wurde der Assay in Cocktail A und B aufgetrennt. Cocktail A beinhaltete Phenacetin, Coumarin, Diclofenac, Dextromethorphan und Testosterone und Cocktail B Bupropion, Amodiaquine, Omeprazole und Chlorzoxazone. Unter Verwendung dieser beiden Sets waren die bestimmten IC_{50} Werte vergleichbar mit den Werten aus einem Single-Assay und mit denen der Literatur. Zusammenfassend war der neuen Assay in der Lage valide, reproduzierbare und verwendbare Ergebnisse zu liefern. Die getesteten Missbrauchsdrogen zeigten alle eine Hemmung gegen mindestens ein Isoenzym. Die Methylendioxy Designer-Drogen hemmten CYP2D6 mit Werten vergleichbar mit Paroxetin oder Fluoxetin. Die Methylenedioxy-Ethylphenethylamine zeigten das höchste Potenzial gegen CYP2D6. Die Cathinone zeigten ebenfalls eine Inhibition gegen CYP2B6 vergleichbar zu Efavirenz. Insgesamt wurden CYP1A2, CYP2A6, CYP2B6, CYP2D6 und CYP3A vergleichbar zu bekannten Hemmstoffen gehemmt. Die getesteten DALTs zeigten die stärkste Hemmung gegen CYP1A2, CYP2D6 und CYP3A wie bekannte Hemmstoffe. Die CYP1A2 Hemmung von 5-MeO-DALT konnte auch in vivo mit Ratten gezeigt werden.