CHARLES UNIVERSITY
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BIOTRANSFORMATION OF PLANT SECONDARY METABOLITES AND THEIR MODULATORY EFFECTS ON DRUG-METABOLIZING ENZYMES

Dissertation

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Hradec Králové 2024

“An expert is a person who has made all the mistakes
that can be made in a very narrow field.”

- Niels Bohr

STATEMENT OF AUTHORSHIP

I hereby declare that this thesis is my original work which I solely composed by myself under the supervision of Assoc. Prof. PharmDr. Iva Boušová, Ph.D. All used literature and other sources are summarized in the list of references and properly cited. This work has not been submitted for any different or equal degree.

Prohlašuji, že tato práce je mým původním autorským dílem, které jsem vypracovala samostatně pod vedením své školitelky doc. PharmDr. Ivy Boušové, Ph.D. Veškerá literatura a další zdroje, z nichž jsem při zpracování čerpala, jsou uvedeny v seznamu použité literatury a v práci řádně citovány. Práce nebyla využita k získání jiného nebo stejného titulu.

In Hradec Králové Mgr. Michaela Šadibolová

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ABSTRACT

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Candidate: Mgr. Michaela Šadibolová
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Title of Dissertation: Biotransformation of plant secondary metabolites and their modulatory effects on drug-metabolizing enzymes

Sesquiterpenes and prenylflavonoids represent two classes of plant secondary metabolites that form an inherent part of human diet. They exert many beneficial biological activities, and accordingly, they are used as active constituents of herbal products. However, as xenobiotics, they also interact with a battery of drug-metabolizing enzymes and can in turn modulate their activity and/or expression, which may lead to herb-drug interactions. Sesquiterpenes and prenylflavonoids mentioned in this dissertation have been under-researched in this aspect.

Three acyclic sesquiterpenes (farnesol, *cis*-nerolidol, *trans*-nerolidol) and three cyclic sesquiterpenes (α-humulene, β-caryophyllene, β-caryophyllene oxide) activated gene transcription mediated by pregnane X receptor; however, they did not alter the mRNA or protein expression of their downstream targets, namely cytochrome P450 (CYP) 3A4 and CYP2C, neither did they affect the expression of the main hepatic carbonyl reducing enzymes.

Sesquiterpenes are substrates of drug-metabolizing enzymes which modify their structures in order to facilitate their excretion. In particular, a sesquiterpene lactone helenalin underwent two types of biotransformation reactions, oxidation and reduction. The former was catalyzed by a number of human CYP enzymes. Amongst them, CYP2A13 exhibited the highest efficiency, but it was in turn inactivated in a mechanism-based manner. In addition, a competitive inhibition of CYP3A4 by helenalin was also observed.

Intestinal drug-metabolizing enzymes are often neglected despite the fact that they largely contribute to the first-pass elimination of orally administered drugs and xenobiotics. Yet, prenylflavonoids xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin significantly increased the activity of glutathione-*S*-transferase, catechol-*O*-methyltransferase, and the mRNA expression of uridine diphosphate-glucuronosyltransferase 1A6, while they decreased the activity of sulfotransferase. Collectively, we discovered that some of the studied compounds might pose a risk of provoking herb-drug interactions.

ABSTRAKT

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Názov dizertačnej práce: Biotransformácia sekundárnych rastlinných metabolitov a ich modulačné účinky na enzýmy metabolizujúce liečivá

Seskviterpény a prenylované flavonoidy predstavujú dve triedy rastlinných sekundárnych metabolitov tvoriace prirodzenú súčasť ľudskej stravy. Majú mnoho prospešných biologických účinkov a sú podľa toho využívané ako aktívne súčasti rastlinných prípravkov. Ako xenobiotiká ale interagujú s množstvom enzýmov metabolizujúcich liečivá, môžu obratom meniť ich aktivitu a/alebo expresiu, čo môže následne vyústiť do liekových interakcií. Seskviterpény a prenylflavonoidy zmienené v tejto dizertácii sú v tomto aspekte málo preskúmané.

Tri acyklické seskviterpény (farnezol, *cis*-nerolidol, *trans*-nerolidol) a tri cyklické seskviterpény (α-humulén, β-karyofylén, β-karyofylénoxid) aktivovali génovú expresiu sprostredkovanú pregnanovým X receptorom, no nespôsobili významné zmeny v mRNA alebo proteínovej expresii jeho cieľových génov, cytochrómu P450 (CYP) 3A4 a CYP2C, a neovplyvnili ani hlavné pečeňové karbonyl redukujúce enzýmy.

Seskviterpény sú substrátmi enzýmov metabolizujúcich liečivá, ktoré modifikujú ich štruktúru s cieľom uľahčenia ich následnej eliminácie. Seskviterpénový laktón helenalín podstúpil dva typy biotransformačných reakcií, oxidáciu a redukciu. Oxidácia bola katalyzovaná niekoľkými ľudskými CYP. Z nich bola najefektívnejšia izoforma CYP2A13, ktorá bola ale obratom ireverzibilne inaktivovaná mechanizmom tvorby reaktívneho medziproduktu/metabolitu. Taktiež bola pozorovaná aj kompetitívna inhibícia CYP3A4.

Črevné enzýmy metabolizujúce liečivá sú často prehliadané aj napriek tomu, že sa výrazne podieľajú na presystémovej eliminácii perorálne prijatých liečiv a xenobiotík. Prenylované flavonoidy xantohumol, izoxantohumol, 8-prenylnaringením a 6-prenylnaringenín však značne zvýšili aktivitu glutatión-*S*-transferázy a katechol-*O*-metyltransferázy, mRNA expresiu uridíndifosfát-glukuronozyltransferázy a popritom znížili aktivitu sulfotransferázy. Celkovo sme teda zistili, že niektoré zo študovaných zlúčenín môžu predstavovať riziko z hľadiska rozvoja liekových interakcií.

TABLE OF CONTENTS

STATEMENT OF AUTHORSHIP i

ACKNOWLEDGEMENTS ii

ABSTRACT iii

ABSTRAKT iv

1 INTRODUCTION 1

2 THEORETICAL BACKGROUND 2

2.1 Drug-metabolizing enzymes 2

2.1.1 Phase I drug-metabolizing enzymes 4

2.1.2 Phase II drug-metabolizing enzymes 9

2.2 Modulation of drug-metabolizing enzymes by xenobiotics 15

2.2.1 Enzyme induction 15

2.2.2 Enzyme inhibition 19

2.2.3 Consequences of enzyme induction and inhibition 21

2.3 Natural compounds as modulators of drug-metabolizing enzymes 23

2.3.1 Sesquiterpenes 23

2.3.2 Prenylflavonoids 27

3 AIMS OF THE DISSERTATION 29

4 PUBLICATIONS INCLUDED IN THE DISSERTATION WITH CANDIDATE'S CONTRIBUTION 30

4.1 Publication I 30

4.2 Publication II 30

4.3 Publication III 31

5 RESULTS 32

5.1 Sesquiterpenes and hepatic phase I drug-metabolizing enzymes 32

5.1.1 Modulation of mRNA and protein expression 32

5.1.2 Biotransformation and inhibitory activity toward cytochromes P450 33

5.2 Prenylflavonoids and intestinal phase II drug-metabolizing enzymes 35

5.2.1 Modulation of enzyme activity and mRNA expression 35

6 DISCUSSION 36

7 CONCLUSIONS 40

8 DISSEMINATION OF RESEARCH FINDINGS 41

8.1 Presentations related to the topic of the dissertation 41

8.2 Publications unrelated to the topic of the dissertation 41

8.3 Presentations unrelated to the topic of the dissertation 42

8.4 Grant projects 42

8.5 International scientific experience 43

8.6 Additional education 43

8.7 Teaching experience 44

9 LIST OF ABBREVIATIONS 45

10 REFERENCES 46

11 APPENDICES 59

1 INTRODUCTION

Herbal products have long been used as part of complementary and alternative medicine; in addition, an increasing trend regarding their use across the world has also been observed (Ekor, 2014; WHO, 2021). It has been estimated that the retail sales of herbal products and supplements in the United States surpassed $11.2 billion for the first time in 2020 and increased by 17.3% in comparison with previous year (Smith et al., 2021). Herbal products are often regarded by consumers as safe, balanced, and moderate approach to addressing different health issues. However, their growing popularity goes hand in hand with an increasing number of people using herbal products concomitantly with conventional medicines, which may lead to unintended and potentially deleterious herb-drug interactions (Ekor, 2014; WHO, 2021). Elderly people are particularly at risk as they are more likely to suffer from multiple comorbidities and participate in polypharmacy (Agbabiaka et al., 2017).

Herb-drug interactions may occur at both pharmacodynamic and pharmacokinetic level. The former change the biological/pharmacological effect of concomitantly used prescription drugs, while the latter change their absorption, distribution, metabolism, or excretion. The major underlying mechanism of pharmacokinetic herb-drug interactions is the modulation of the expression and/or activity of drug-metabolizing enzymes (DMEs), which serve as a means of protection against oxidative and chemical stress. The modulation of DMEs occurs via two distinct mechanisms with different clinical implications. Enzyme induction leads to the upregulation of the expression of DMEs and thus increased clearance of co-administered drugs, whereas enzyme inhibition reduces their catalytic activity, which may result in the accumulation of active drugs and manifestation of their toxic effects (Fasinu et al., 2012; WHO, 2021). Of particular importance are herb-drug interactions mediated by CYPs and uridine diphosphate-glucuronosyltransferases (UGTs), as they metabolize a vast majority of currently used prescription drugs (Hakkola et al., 2020; Liu et al., 2019; Rowland et al., 2013).

The evaluation of herb-drug interactions is challenging due to the complexity of herbal products, variability of their composition, uncertainty of the causative constituents, and lack of data regarding constituents' pharmacokinetics. In addition, a standardization of the prediction and evaluation of herb-drug interactions has not yet been established (WHO, 2021). This dissertation thus presents the results of biotransformation as well as modulatory effects of selected components of herbal products on phase I and phase II DMEs.

2 THEORETICAL BACKGROUND

2.1 Drug-metabolizing enzymes

Throughout its life, human organism is exposed to a myriad of different chemical compounds of either natural or synthetic origin with no physiological functions *per se*. Yet, these exogenous compounds, also referred to as *xenobiotics,* may still provoke a wide range of biological effects upon exposure. As a means of protection, human organism (as well as other living organisms) has developed a complex system of defense mechanisms which aim to limit the uptake and distribution of xenobiotics as well as to reduce their toxicity and facilitate their excretion (Ioannides, 2001).

DMEs play a crucial role in the *detoxification* of xenobiotics; in addition, they also participate in the metabolism of endogenous compounds, such as cholesterol, steroid hormones, bilirubin, and many others (Cederbaum, 2015; Ioannides, 2001). Using an array of biotransformation reactions, DMEs alter the chemical structure of xenobiotics and as a result, produce metabolites with increased hydrophilicity and reduced biological activity that are readily excreted. Metabolic detoxification is essential especially in the case of highly lipophilic xenobiotics that can easily pass through cell membranes by passive diffusion and could otherwise accumulate to toxic levels (Coleman, 2020; Ioannides, 2001). Paradoxically, the activity of certain classes of DMEs, namely CYPs, aldo-keto reductases (AKRs), *N*-acetyltransferases, sulfotransferases (SULTs), and peroxidases, may yield highly reactive metabolites that are able to irreversibly interact with biomacromolecules, such as DNA, RNA, or proteins, change their function and provoke different types of toxicity. This process is also known as *bioactivation*, and it has been implicated as an underlying cause of adverse drug reactions and procarcinogen activation (Ioannides, 2001; Rendic & Guengerich, 2012; Walsh & Miwa, 2011). The knowledge about the mechanisms of biotransformation reactions catalyzed by DMEs has also been employed in the area of drug design and development. Inactive prodrugs which are designed to possess better physicochemical and pharmacokinetic properties are metabolized by DMEs *in vivo* into their active forms that can exert intended pharmacological effects (Rautio et al., 2008).

DMEs are classified into three main groups based on the character of reactions they catalyze. *Phase I* DMEs are responsible for unmasking or *de novo* addition of polar functional groups, such as -OH, $-NH_2$, -SH, or -COOH, within the molecule of a xenobiotic. The usual

phase I reactions include hydroxylation, *N*- and *O*-dealkylation, *N*-, *S*-, and *P*-oxidation, oxidative deamination and dehalogenation, reduction, or hydrolysis. As a result, metabolites formed during phase I are more polar and possess functional groups that enable them to undergo conjugation reactions catalyzed by *phase II* DMEs. During phase II, xenobiotics are conjugated with endogenous substrates, such as glucuronic acid, glutathione, sulphate, or amino acids, through the activity of various transferases. These conjugation reactions also increase the polarity and hydrophilicity of xenobiotics and thus facilitate their excretion (Croom, 2012; Ioannides, 2001). However, polar character of xenobiotics or their metabolites abolishes their ability to pass through cell membranes by passive diffusion. It is thus the role of *phase 0/phase III* DMEs to actively transport them in and out of cells into the interstitial fluid, bloodstream, kidneys, or bile canaliculi (Anzenbacher & Zanger, 2012; Coleman, 2020; Prakash et al., 2015). Based on the direction of transport, two major superfamilies of drug transporters can be distinguished – efflux transporters from the ATP-binding cassette (ABC) superfamily that pump xenobiotics out of cells and uptake transporters from the solute carrier superfamily, which transport xenobiotics into cells (Cobbina & Akhlaghi, 2017).

DMEs are expressed in many different tissues and organs within a human organism. The expression and activity of DMEs are characteristic for high interindividual and intraindividual variability influenced by genetic predisposition, age, sex, presence of a disease, individual's diet, pregnancy, and many other factors (Cederbaum, 2015). The highest expression of DMEs is nevertheless found in the liver and intestines making them the central organs of biotransformation. In the intestines, the highest levels of DMEs have been reported in jejunum and duodenum followed by ileum and colon (Doherty & Charman, 2002; Drozdzik et al., 2018; Fritz et al., 2019). As oral administration represents the major route of exposure to xenobiotics, both the liver and intestines contribute significantly to their presystemic elimination (*first-pass effect*) affecting their bioavailability, disposition, and biological activity (Almazroo et al., 2017; Drozdzik et al., 2018). Yet, they are thus more susceptible to the development of drug-induced injury (Cederbaum, 2015; Navarro & Senior, 2006; Pusztaszeri et al., 2007).

The following chapters are dedicated to selected phase I and phase II DMEs which expression and/or activity was studied in this dissertation.

2.1.1 *Phase I drug-metabolizing enzymes*

Cytochromes P450

CYPs constitute a major family of heme-containing oxidases found in bacteria, yeast, plants, invertebrates, and vertebrates that catalyze primarily the oxidation of a wide range of endogenous as well as exogenous substrates (xenobiotics). Human CYPs are localized predominantly in the endoplasmic reticulum and participate in the metabolism of cholesterol and bile acids, prostaglandins, vitamins A and D, or steroid hormones, such as testosterone, progesterone, and cortisol. Apart from that, CYPs can also metabolize a wide range of structurally diverse xenobiotics which makes them an outstanding class of DMEs. It has been estimated that they contribute to the metabolism of approximately 75% of drugs in medical use (Guengerich, 2006; Nebert & Russell, 2002; Neve & Ingelman-Sundberg, 2008).

Nonetheless, due to the nature of their catalytic activity, CYPs are also greatly responsible for the bioactivation of xenobiotics into reactive intermediates and the production of reactive oxygen species (Cederbaum, 2015; Rendic & Guengerich, 2012; Walsh & Miwa, 2011). Following substrate binding, NADPH-P450 reductase reduces Fe^{3+} in the heme moiety of the CYP structure into Fe^{2+}, which facilitates binding of an oxygen molecule and formation of a CYP-FeO_2^{2+} complex. Subsequently, another electron is donated by either the activity of NADPH-P450 reductase or cytochrome b5, the resulting complex is protonated, and a molecule of water is released. Eventually, an oxygen atom is incorporated into the substrate's structure, and a modified substrate is released (Anzenbacher & Zanger, 2012).

CYPs are ubiquitously expressed in various organs and tissues, namely the liver, intestines, kidneys, lungs, brain, heart, skin, placenta, endothelium, or spleen (Anzenbacher & Anzenbacherova, 2001). Human genome contains 57 functional genes for CYPs; based on the percentage of shared sequence identity, they are then divided into 18 families and 44 subfamilies. Out of these 18 families, only three have been reported to take part in the biotransformation of drugs and xenobiotics, namely CYP1, CYP2, and CYP3. Interestingly, they largely overlap in their substrate specificity which also likely contributes to the manifestation of herb-drug interactions. The expression of CYPs is highly variable in between individuals; several factors including loss-of-function and gain-of-function genetic polymorphism, epigenetic regulation, sex, age, and the presence of an illness or an inflammatory condition affect their expression (**Figure 1**). Furthermore, majority of drug-metabolizing CYPs are inducible by their substrates or other xenobiotics through the activation

of ligand-activated transcription factors, mainly pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR) (**Chapter 2.2.1**) (Cederbaum, 2015; Zanger & Schwab, 2013).

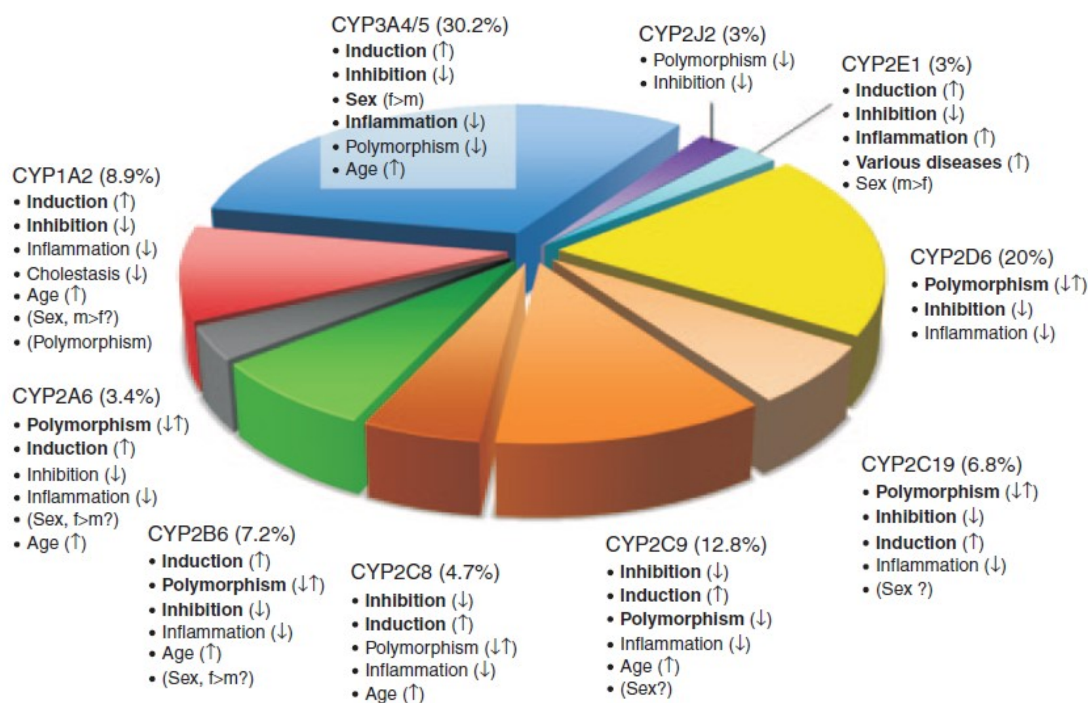

Figure 1 The contribution of individual CYPs to the biotransformation of prescription drugs in clinical use and factors influencing their expression (Zanger & Schwab, 2013).

CYP1 family encompasses three isoforms - CYP1A1, CYP1A2, and CYP1B1, which expression is inducible predominantly through the activation of AhR pathway. CYP1A1 and CYP1B1 are expressed mainly in extrahepatic tissues and involved in the bioactivation of environmental pollutants, such as polycyclic aromatic hydrocarbons (PAH), arylamines, or heterocyclic aromatic amines found in charbroiled meat (Coleman, 2020; Falero-Perez et al., 2018; Walsh et al., 2013). Together with CYP1A2, they account for 48% of all carcinogen activation reactions mediated by CYPs (Rendic & Guengerich, 2012). The inhibition of their catalytic activity may have a chemopreventive effect; several natural compounds including plant flavonoids with antitumor activity act as inhibitors of CYP1 (Badal & Delgoda, 2014; Baer-Dubowska & Szaefer, 2013; D'Uva et al., 2018). CYP1A2 is expressed predominantly in the liver, and although it is also implicated in the bioactivation of PAH and other carcinogens (Rendic & Guengerich, 2012), it is more relevant to the metabolism of prescribed drugs than the other two CYP1 isoforms. CYP1A2 is involved in the clearance of antipsychotics olanzapine and clozapine, antidepressants fluvoxamine, imipramine, and clomipramine,

analgesics and antipyretics acetaminophen and naproxen, and many others (Anzenbacher & Anzenbacherova, 2001; Zanger & Schwab, 2013). AhR ligands, such as PAH, polychlorinated biphenyls, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a known human carcinogen, are known inducers of CYP1A2; the induction by carbamazepine, phenobarbital, omeprazole, ritonavir, phenytoin, or isothiocyanates found in cruciferous vegetable was also described. On the other hand, the activity of CYP1A2 is inhibited by α-naphthoflavone, fluvoxamine, ciprofloxacin, or disulfiram (Cederbaum, 2015; Eagles et al., 2020; Zanger & Schwab, 2013).

CYP2 family is the largest family of CYPs in mammals responsible for the metabolism of approximately half of prescribed drugs. Individual isoforms are transcriptionally regulated by different nuclear factors including mainly PXR and CAR, and to a lesser extent glucocorticoid receptor, vitamin D receptor, or estrogen receptor (Nebert & Russell, 2002; Zanger & Schwab, 2013). The subfamily CYP2A contains two isoforms, CYP2A6 and CYP2A13. CYP2A6 is a liver-specific enzyme involved in the metabolism of coumarin and several prescribed drugs like disulphiram, halothane, or valproic acid. It is the main isoform responsible for the bioactivation of the antineoplastic prodrug tegafur into its active form, 5-fluorouracil, and it metabolizes nicotine into its inactive form cotinine (Coleman, 2020; Zanger & Schwab, 2013). The inhibition of CYP2A6 slows down the deactivation of nicotine and increases its plasma level; it is thus implicated as a potential strategy for smoking cessation (Raunio & Rahnasto-Rilla, 2012). CYP2A13 is expressed in the respiratory tract and shares some substrate specificity with CYP2A6. However, it is largely involved in the bioactivation of a tobacco procarcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Su et al., 2000). CYP2B6 is a minor hepatic yet highly polymorphic isoform involved in the biotransformation of efavirenz, bupropion, artemisinin, propofol and ketamine, and it plays an important role in the activation step of a prodrug cyclophosphamide (Zanger & Schwab, 2013). Of the CYP2C subfamily, three isoforms are relevant for the biotransformation of xenobiotics – CYP2C8, CYP2C9, and CYP2C19. All isoforms are expressed predominantly in the liver, the highest protein amount has been reported for CYP2C9. CYP2C9 is also highly expressed in the intestines (Drozdzik et al., 2018; Fritz et al., 2019; Zanger & Schwab, 2013). Regarding transcriptional activation, CYP2C8 is deemed to be the most inducible CYP2C isoform. Interestingly, despite sequence similarity, the substrate specificity of CYP2C isoforms does not overlap largely. CYP2C9 metabolizes most nonsteroidal anti-inflammatory drugs, oral antidiabetics glibenclamide and glimepiride, and warfarin; CYP2C8 metabolizes antidiabetic thiazolidinediones, amiodarone, and paclitaxel; CYP2C19 detoxifies most proton pump inhibitors, antidepressants like imipramine and venlafaxine, or clopidogrel (Coleman, 2020; Zanger & Schwab, 2013).

CYP2D6 is a minor hepatic isoform with significant interindividual variability of protein expression due to genetic polymorphism. However, considering its contribution to the biotransformation of xenobiotics, it is the second most effective drug-metabolizing CYP detoxifying approximately 15-25% of prescribed drugs from different therapeutic classes, such as antidepressants, antipsychotics, beta-blockers, antiarrhythmics, analgesics, or antineoplastic agents. The modulation of CYP2D6 by drugs or other xenobiotics might have severe implications regarding adverse drug reactions or treatment outcomes. Surprisingly, little is known about the transcriptional regulation of CYP2D6, and it seems that this isoform is probably non-inducible (Coleman, 2020; Zanger & Schwab, 2013). Last but not least, hepatic CYP2E1 metabolizes low molecular weight compounds, such as ethanol, acetone, or anesthetics halothane and isoflurane; it is also involved in acetaminophen-induced hepatotoxicity. The catalytic activity of CYP2E1 yields reactive oxygen species, namely superoxide anion radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), which play an essential role in the pathophysiology of CYP2E1-mediated liver injury. Furthermore, CYP2E1 is implicated in the development of hepatocellular carcinoma as it is able to activate several procarcinogens (Harjumaki et al., 2021; Zanger & Schwab, 2013).

CYP3 family includes only two isoforms that are involved in the metabolism of xenobiotics, CYP3A4 and CYP3A5. Yet, CYP3A4 is the most abundant CYP in the liver and intestines contributing to the metabolism of astonishing 30-50% of all prescription drugs. The modulation of its activity might therefore have significant toxicological consequences. CYP3A4 metabolizes a broad range of structurally very diverse drugs from numerous therapeutic classes. The expression of CYP3A4 is quite complex, and it is regulated through a number of transcription factors including PXR and CAR, but also vitamin D receptor, glucocorticoid receptor, farnesoid X receptor, or liver X receptor. Several drugs as well as natural compounds have been found to induce its expression or inhibit its activity. Drugs and plant products like rifampicin, phenytoin, phenobarbital, dexamethasone, carbamazepine, or hyperforin from *Hypericum perforatum* L. (St. John's Wort) belong among the well-known inducers of CYP3A4. On the other hand, protease inhibitors, macrolide antibiotics, ketoconazole, or citrus juices are listed as potent CYP3A4 inhibitors. In the same vein, CYP3A5 is transcriptionally regulated by PXR and CAR. In addition, its expression is subjected to genetic polymorphism with low expression among Caucasian population. Even though CYP3A4 and CYP3A5 share high sequence similarity and also overlap in substrate specificity, it is the expression of CYP3A5 that largely affects the pharmacokinetics, therapeutic outcomes, and toxicity of the immunosuppressive agent tacrolimus (Coleman, 2020; Zanger & Schwab, 2013).

Carbonyl reducing enzymes

Carbonyl reducing enzymes include two enzyme superfamilies, AKRs and short-chain dehydrogenases/reductases (SDRs). Both groups of reductases are soluble cytosolic NAD(P)(H)-dependent oxidoreductases ubiquitously expressed in nearly all phyla, albeit some SDRs are also found in endoplasmic reticulum. Their main function is to catalyze the reduction of carbonyl-containing aldehydes, ketones, and quinones into their corresponding alcohols and hydroquinones. Carbonyl reducing enzymes possess broad and overlapping substrate specificity metabolizing endogenous compounds, such as steroids, prostaglandins, retinoids, sugar aldehydes, or lipid peroxidation byproducts, as well as xenobiotics (Hoffmann & Maser, 2007; Malatkova et al., 2010; Penning, 2015).

The nomenclature of AKRs is based on the shared sequence similarity in a manner similar to CYPs. The superfamily of human AKRs embraces 15 isoforms from 3 families and 7 subfamilies. Two families, AKR1 and AKR7 have been suggested to participate in the metabolism of xenobiotics (Amai et al., 2020; Fukami et al., 2022). For SDRs, 75 isoforms have been found in humans that are classified into 47 families designated by a letter indicating the type of SDR – C (classical), E (extended), A (atypical), I (intermediate), D (divergent), X (complex), or U (unassigned). Only six SDRs are deemed to be involved in the biotransformation of xenobiotics, among them carbonyl reductase 1 (CBR1/SDR21C1) (Amai et al., 2020; Malatkova & Wsol, 2014).

Four isoforms of the AKR1C subfamily (AKR1C1-AKR1C4, also known as hydroxysteroid dehydrogenases) and CBR1 seem to be the most abundant hepatic isoforms. Apart from AKR1C4, these enzymes are also expressed extrahepatically (Amai et al., 2020; Kassner et al., 2008). In line with their role as defense mechanisms against oxidative stress, they contain several antioxidant response elements (ARE) in their promotor region and are therefore inducible through cytoprotective Kelch-like ECH-associated protein 1 (Keap1)/nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. In addition, the transcriptional regulation of CBR1 is more complex and involves also glucocorticoid receptor and activator protein-1. The latter is known to bind to a sequence motif embedded within ARE (Ebert et al., 2016; Penning et al., 2019). Carbonyl reducing enzymes, especially CBR1 and AKR1C3 are largely involved in the deactivation of antineoplastic agents, such as doxorubicin and daunorubicin, yielding less active alcohols doxorubicinol and daunorubicinol, respectively. Reduced metabolites, however, demonstrate higher toxicity and are implicated in the development of chronic cardiomyopathy, a hallmark adverse effect of anthracycline treatment (Barski et al., 2008; Bukum et al., 2019;

Kassner et al., 2008). In addition, the overexpression of CBR1 and AKR1C has been observed in a number of anthracycline-resistance tumor cells. In order to overcome the anthracycline resistance and cardiotoxicity, selective inhibitors of these enzymes are being tested (Bukum et al., 2019; Hofman et al., 2014; Mordente et al., 2015). Apart from the antineoplastic agents, AKR1C and CBR1 participate in the biotransformation of many clinically used drugs. AKR1C1 and AKR1C2 share 97.8% sequence similarity and therefore overlap largely in substrate specificity metabolizing dolasetron, oxcarbazepine, and pentoxifylline. AKR1C3 and CBR1 metabolize acetohexamide, loxoprofen (together with AKR1C4), whereas AKR1C4 is involved in the biotransformation of naloxone (Fukami et al., 2022).

AKR1C isoforms catalyze the bioactivation of PAH-diols (metabolites of CYP1) into reactive *o*-quinones and are thus also involved in PAH-induced toxicity. On the other hand, the reduction of NNK to nicotine-derived nitrosamino-alcohols by CBR1 and AKR1C represents a major route of detoxication of NNK that is otherwise bioactivated by CYP2A (Jin & Penning, 2007; Shimada, 2006).

2.1.2 Phase II drug-metabolizing enzymes

Glutathione-*S*-transferases

Glutathione-*S*-transferases (GSTs) are ubiquitously present in all cellular life forms, and they are vital for maintaining cell homeostasis. Based on their subcellular localization, they are divided into three families – cytosolic, mitochondrial, and membrane-bound GSTs. As for this dissertation, only cytosolic GSTs will be further discussed. In humans, cytosolic GSTs are expressed in most tissues and organs. They are divides into seven classes, each assigned with a Greek letter, namely alpha (A), mu (M), pi (P), sigma (S), theta (T), zeta (Z), and omega (O). They usually exist as homodimers, albeit some isoforms can form intraclass heterodimers. Heterodimerization occurs between GSTA1 and GSTA2 and between GSTM1 and GSTM2. Interestingly, cytosolic GSTs can account for up to 10% of soluble proteins in human liver. GSTs conjugate a wide range of endogenous and xenobiotic compounds, including byproducts of lipid metabolism and other products of oxidative stress, environmental pollutants and their toxic metabolites produced by CYPs, antineoplastic agents, and many other prescription drugs. Substrates of GSTs usually contain electrophilic moieties which are conjugated to a tripeptide glutathione and subsequently converted to mercapturic acids to aid their excretion (Board & Menon, 2013; Singh & Reindl, 2021). Apart from the conjugation activity of GSTs, some

isoforms also exhibit mild selenium-independent glutathione peroxidase activity and the ability to catalyze the reduction of organic hydroperoxides into their corresponding alcohols (Allocati et al., 2018; Hayes et al., 2005). On the other hand, GSTs are implicated to play a role in the pathogenesis of neurodegenerative and malignant diseases as well as in chemoresistance (Allocati et al., 2018; Singh & Reindl, 2021).

Glutathione is an essential cellular antioxidant playing a multifaceted role in cells. It maintains cellular redox status, participates in degradation of reactive oxygen and nitrogen species, regulates gene transcription and apoptosis, and detoxifies xenobiotics (Jefferies et al., 2003). It is typically present in high concentrations ranging from 0.1 mM to even 10 mM, hence it is the most abundant cellular thiol (Anderson, 1998). However, fasting, excessive alcohol intake, or some prescription drugs can all deplete glutathione levels (Choi et al., 2000; Moine et al., 2018; Vogt & Richie, 1993).

There are five human genes for alpha class of GSTs coding for five GSTA isoforms; however, GSTA1 and GSTA2 are the most abundant enzymes with a very high expression in the liver, intestines, kidneys, or adrenal glands (Mohana & Achary, 2017). The two GSTA enzymes are present as homodimers GSTA1-1 and GSTA2-2, or as a heterodimer GSTA1-2. As for their physiological functions, GSTA1-1 binds bilirubin, steroid hormones, but also various xenobiotics via both covalent and noncovalent bond and thus participates in their intracellular transport, sequestration, and disposition. In addition, together with GSTA2-2 and GSTP1-1, they also participate in the synthesis and modification of leukotrienes and prostaglandins (Board & Menon, 2013; Hayes et al., 2005). GSTA1-1 regulates apoptosis by inhibiting c-Jun *N*-terminal kinase (JNK) signaling via direct protein-protein interaction. As a result, the overexpression of GSTA1-1 inhibits phosphorylation of c-Jun by JNK and activation of caspase-3, maintains the levels of anti-apoptotic B-cell lymphoma 2 and thus confers cancer cells protection against cell death (Romero et al., 2006; Sharma et al., 2006). Contrarily, the overexpression of GSTA1 in human hepatocellular carcinoma cells increased the activity of 5′-adenosine monophosphate-activated protein kinase (AMPK), thereby inhibited the mammalian target of rapamycin (mTOR) signaling, and eventually impaired cancer cell proliferation (Liu et al., 2020).

In the case of pi class, there is only one single functional gene encoding for GSTP1; therefore, GSTP exists as a homodimer GSTP1-1. GSTP1 can also exist as a monomer, and a certain ratio of monomeric and dimeric GSTP1-1 is maintained in the cell (Board & Menon, 2013). GSTP1-1 is involved in enzyme-mediated post-translational protein modification of exposed cysteine residues by *S*-glutathionylation, which occurs as a response mechanism to

oxidative stress. *S*-Glutathionylation concerns various enzymes, transcription factors, or oncogenes and modulates their catalytic activities (Singh & Reindl, 2021). As a monomer, GSTP1 binds to JNK via protein-protein interaction and suppresses the downstream signaling by preventing its phosphorylation. In the state of increased oxidative stress, GSTP1 dissociates from the complex with JNK and forms a homodimer. JNK can therefore undergo phosphorylation and initiate cell proliferation or apoptosis pathways depending on the amount of reactive oxygen species present (Adler et al., 1999; Wang et al., 2001). GSTP1 also inhibits tumor necrosis factor receptor-associated factor 2 and AMPK/mTOR signaling pathways, which ultimately confers resistance and improves survival of cancer cells (Singh & Reindl, 2021). For that reason, different types of GSP1-1 inhibitors are being tested with the aim to overcome chemoresistance, elevate the amount of reactive oxygen species and increase oxidative stress, activate apoptotic cascades, and eventually impair cancer cell survival. Natural compounds provided a couple of structures with such inhibitory activity, namely piperlongumine from *Piper longum* L. that hydrolyzes to hydroxypiperlongumin, subsequently binds to GSTP1, and elevates the level of reactive oxygen species, or curcumin from *Curcuma longa* L. (Board & Menon, 2013; Singh & Reindl, 2021).

UDP-glucuronosyltransferases

UGTs are a superfamily of membrane-bound enzymes present in many phyla, from bacteria to animals. They catalyze covalent addition of an activated uridin-5′-diphospho (UDP)-sugar to an acceptor functional group with a nucleophilic atom, such as N, O, S, or C, via second order nucleophilic substitution mechanism (Jancova et al., 2010; Meech et al., 2019). In human, four families of UGTs (UGT1, UGT2, UGT3, and UGT8) have been described that are further grouped into five subfamilies (UGT1A, UGT2A, UGT2B, UGT3A, and UGT8A) embracing 22 individual UGT isoforms. While the members of UGT1 and UGT2 families play a crucial role in the biotransformation of xenobiotics and environmental pollutants as well as endogenous molecules, such as bilirubin, steroid and thyroid hormones, fat-soluble vitamins, or bile acids, the functions of UGT3 and UGT8 have only recently been discovered and lie predominantly in the regulation of endogenous metabolism rather than in the detoxification of xenobiotics. Interestingly, UGT1 and UGT2 utilize primarily UDP-α-D-glucuronic acid for conjugation, whereas UGT3 and UGT8 prefer UDP-*N*-acetylglucosamine, UDP-glucose, UDP-xylose, or UDP-galactose, which might also be related to their different affinity towards the conjugation of xenobiotics (Meech et al., 2019).

UGT1A and UGT2B isoforms are notable for broad substrate specificity metabolizing approximately 40-70% of clinically used drugs and herbal medicines (Liu et al., 2019). They are also highly expressed in first-pass organs, such as the liver and intestines, and thus significantly affect the bioavailability of orally administered xenobiotics (Jancova et al., 2010; Oda et al., 2015). On the other hand, UGT2A are expressed mainly extrahepatically and involved in the detoxification of PAH metabolites, albeit less attention has been paid to characterize their catalytic activities (Bushey et al., 2013). Similarly to CYPs, UGT1A and UGT2B are inducible (though sometimes repressed) via the activity of several ligand-activated transcription factors, including but not limited to PXR, CAR, AhR, or Nrf2. Due to several factors, namely genetic polymorphism, epigenetic regulation, and already mentioned ligand-activated transcriptional regulation, the expression of UGT1A and UGT2B varies significantly between individuals and contributes to differences in xenobiotic/drug clearance. Furthermore, a link between loss-of-function genetic polymorphisms of a number of UGTs and a higher risk of tumorigenesis has been repeatedly suggested (Meech et al., 2019; Oda et al., 2015). Generally, glucuronidation yields more hydrophilic and often inactive metabolites; however, a specific type of bioactivation is characteristic also for UGTs – the formation of acyl glucuronides able to bind to cell nucleophiles (Oda et al., 2015; Walsh & Miwa, 2011).

UGT1A6 is widely expressed in a number of tissues including the liver, intestines, or brain. Interestingly, serotonin has been found to be a selective endogenous substrate for UGT1A6; therefore, this isoform is involved in the regulation of serotonin-mediated signaling in central and enteric nervous system. Besides, UGT1A6 participates in the metabolism of acetaminophen, aspirin, deferiprone, or valproic acid. UGT1A6 is characteristic for its polymorphic expression with several variant alleles existing in the population (Bock & Kohle, 2005; Meech et al., 2019). Of particular clinical importance is the presence of variant allele UGT1A6*4, which has been associated with an increased risk of anthracycline-induced cardiotoxicity in pediatric population. UGT1A6*4 variant shows significant reduction in enzyme activity compared to UGT1A6*1 (wild-type) allele, and genotypic screening is recommended (Aminkeng et al., 2016). UGT1A6 is also of toxicological importance due to its high affinity to planar phenols and arylamines including a number of well-known human carcinogens, such as 2-naphthylamine and 4-aminobiphenyl. Decreased activity of UGT1A6 due to genetic polymorphism has been associated with an increased risk of breast, endometrial, urinary bladder, and lung cancer plausibly due to its role in carcinogen detoxification (Meech et al., 2019).

Sulfotransferases

SULTs constitute a superfamily of soluble cytosolic enzymes catalyzing the transfer of sulfate (SO_3^-) to acceptor substrates via hydroxyl, amine or hydroxylamine functional groups (Chen et al., 2015). SULTs utilize 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as a universal sulfate donor. They usually exist as homodimers and are found across many different phyla (Coughtrie, 2016; Suiko et al., 2017). Human SULTs are grouped into four families (SULT1, SULT2, SULT4, and SULT6) and encompass at least 12 functional enzymes (Isvoran et al., 2022). They play an essential role in the regulation of endocrine signaling and neurotransmitter homeostasis by engaging in the metabolism of thyroid hormones, steroid hormones, and catecholamines (Coughtrie, 2016).

Members of the SULT1 and SULT2 families further participate in the biotransformation of exogenous compounds. Moreover, they are known to exhibit surprisingly broad substrate specificity and overlap in their substrate profiles (Coughtrie, 2016; Chen et al., 2015). In particular, SULT1A1 and SULT1B1 demonstrate the highest affinity toward various xenobiotics, with the former generally possessing lower K_m values (Coughtrie, 2016). Similarly to glucuronidation, sulfate conjugation usually yields water-soluble and less active or even inactive metabolites that are readily excreted. However, in certain cases, *O*-sulfonation bioactivates chemical procarcinogens, namely PAH, heterocyclic amines, benzylic alcohols, or hydroxamic acids, and allows for pro-tumorigenic DNA modification. It has been estimated that SULTs make up the second most frequent group of carcinogen-converting enzymes, with CYPs being top-ranking (Rendic & Guengerich, 2012).

SULT1A1 is the major hepatic SULT isoform with a relatively high expression also in the intestines and other extrahepatic tissues (Riches et al., 2009). It shows high affinity particularly for phenolic compounds, hence its alternative descriptive name phenol sulfotransferase, but also various carcinogenic compounds, such as PAH, benzylic alcohols, or secondary nitroalkanes. From the pharmacological point of view, SULT1A1 detoxifies/activates several drugs, namely acetaminophen, naproxen, minoxidil, morphine, or naloxone (Chen et al., 2015; Isvoran et al., 2022). Variant alleles described for SULT1A1 negatively affect its function and are implicated in breast, endometrial, or colorectal cancer development, although further research is needed (Chen et al., 2015; Isvoran et al., 2022). SULT1A2 shares 96% sequence similarity with SULT1A1, and they also overlap in substrate profiles. However, due to a splicing defect, SULT1A2 is poorly translated in humans and expressed in tiny amounts only in the liver (Isvoran et al., 2022).

Methyltransferases

Methyltransferases allocate a methyl moiety from *S*-adenosyl-L-methionine to a nucleophilic atom (N, O, S, or C) found in the structures of proteins, lipids, polysaccharides, nucleic acids, and various small molecules of endogenous and exogenous origin. Methyltransferases are substantial regulators of gene expression, protein function, or neurotransmitter signaling (Lennard & Wang, 2017). More than 200 methyltransferases have been predicted in human genome, accounting for approximately 0.9% of all gene products; however, only one third of them has been characterized so far (Petrossian & Clarke, 2011). Methyltransferases are usually grouped based on the nucleophile to which they transfer the methyl functional group into *O*-, *N*-, *S*-, and *C*-methyltransferases (Lennard & Wang, 2017).

Catechol-*O*-methyltransferase (COMT), as its name discloses, metabolizes endogenous compounds of catecholamine structure, namely neurotransmitters dopamine, epinephrine, norepinephrine, and structurally related drugs and xenobiotics, such as levodopa, methyldopa, or isoprenaline. COMT further participates in the biosynthesis of melanin, catechol estrogens, and ascorbic acid. In addition, polycyclic catechols, such as tea catechins (-)-epicatechin or (-)-epigallocatechin, as well as xenobiotics with a hydroxybenzoyl functional group or those with phenolic group additionally hydroxylated in *ortho* position by CYPs may also become substrates for COMT. It is ubiquitously expressed in various tissues and functions as a soluble cytosolic or membrane-bound enzyme; the two forms differ by fifty amino acids at the *N*-terminus of the membrane-bound form. The ratio of cytosolic versus membrane-bound forms also differs from tissue to tissue; the soluble form is usually dominating. However, due to its ubiquitous expression, inhibitors of peripheral COMT (along with inhibitors of dopamine decarboxylase) are utilized in the pharmacotherapy of Parkinson's disease to assure that levodopa (a precursor for dopamine) reaches therapeutic concentration in the central nervous system. The activity of COMT is affected by genetic polymorphism, and it has been suggested that interindividual or rather interethnic differences in COMT activity correlate with different frequency of adverse effects caused by the treatment with levodopa (Lennard & Wang, 2017; Zhu, 2002).

2.2 Modulation of drug-metabolizing enzymes by xenobiotics

2.2.1 Enzyme induction

Enzyme induction has evolved as a protective mechanism of living organisms against chemical stress during which xenobiotics upregulate the amount and/or activity of DMEs, which inevitably results in their increased biotransformation and excretion. There are two main mechanisms of xenobiotic-initiated enzyme induction – a more frequent *transcriptional regulation* of enzyme expression controlled by a group of ligand-activated transcription factors and a less frequent *post-transcriptional regulation* mediated via increased mRNA and/or protein stabilization or decreased degradation (Anzenbacher & Zanger, 2012; Hakkola et al., 2020).

Transcriptional mechanism of enzyme induction

Transcriptional mechanism of xenobiotic-initiated enzyme induction is mediated by a group of ligand-activated transcription factors, also known as *xenobiotic-sensing receptors*, which are structurally divided into two groups - nuclear receptors and basic helix-loop-helix Per-Arnt-Single minded (bHLH-PAS) proteins (Hakkola et al., 2020).

Nuclear receptors encompass a large superfamily of 48 different transcription factors involved in the regulation of many physiological processes, from development to homeostasis. This superfamily includes steroid, retinoid, and thyroid hormones receptors, such as PXR, CAR, glucocorticoid receptor, vitamin D receptor, estrogen receptor, farnesoid X receptor, or liver X receptor. However, PXR and CAR are regarded as the primary nuclear receptors involved in the transcriptional regulation of DMEs (Tolson & Wang, 2010). The structural features of nuclear receptors are very similar between different members of the family. They all contain a highly conserved cysteine-rich *N*-terminal DNA-binding domain which binds to the corresponding response elements in the promotor region of target genes via two zinc finger motifs, a less conserved *C*-terminal ligand-binding domain which accommodates various xenobiotic and/or endobiotic substrates, and a hinge region which represents a flexible linkage between the two domains allowing for simultaneous DNA and ligand binding. In addition, their *N*- and *C*-termini harbor two transactivation domains – ligand-independent activation function 1 and ligand-dependent activation function 2, respectively, that guide transcriptional coregulators to the promotor region of target genes (Chen et al., 2012; Rigalli et al., 2021).

PXR, also denoted as NR1I2, plays an essential role in the transcriptional regulation of many DMEs from all phases of drug metabolism. In addition, it has been established that PXR also regulates the expression of genes involved in intermediate metabolism, predominantly in the metabolism of glucose, lipids, cholesterol, or bile acids (Pavek, 2016). PXR is expressed mainly in the liver and intestines, and to a lesser extent in many other tissues and organs. Its cellular localization is still quite debatable; however, current data point to the nuclear localization of human PXR (Rigalli et al., 2021; Wang et al., 2012). There, PXR resides in an inactive form as a complex with corepressors, such as nuclear receptor corepressor 2 or silencing mediator of retinoid and thyroid receptors. Upon ligand binding, the affinity of PXR for corepressor proteins decreases, while the affinity for coactivator proteins, such as members of the steroid receptor coactivator (SRC) family or peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), increases. PXR forms a heterodimer with retinoid X receptor (RXR), and together they bind to xenobiotic responsive elements (XRE) in the promotor region of target genes characterized by direct or everted repeats of the consensus motif AG(G/T)TCA. Gene transcription is initiated by the recruitment of coactivators with histone acetylase activity that modify the chromatin structure and by the assembly of transcription initiation complex (Hakkola et al., 2020; Pavek, 2016; Rigalli et al., 2021; Wang et al., 2012). Unlike other nuclear receptors, PXR has a very bulky and flexible ligand-binding domain which makes it a promiscuous nuclear receptor enabling accommodation of a wide range of structurally diverse substrates including numerous prescription drugs or natural compounds. In addition, the ligand-binding domain of PXR is also less conserved between different species, which may explain species-specific transcriptional response upon the exposure to some xenobiotics (Hakkola et al., 2020). PXR has been found to regulate the expression of human CYP2A6, CYP2B6, CYP2C, CYP3A, AKR1C1/2, UGT1A1, UGT1A3, UGT1A6, SULT2A1, or GSTA1, as well as the expression of drug transporters such as ABCB1 (P-glycoprotein) and multidrug resistance-associated protein (MRP) 2 (Hakkola et al., 2020; Chai et al., 2013; Tolson & Wang, 2010).

CAR, also designated as NR1I3, is also expressed in the liver and intestines. Similarly to PXR, it has long been considered only as a xenobiotic-sensing receptor regulating the expression of CYP2A6, CYP2B6, CYP2C, CYP3A, UGTA1, SULT2A1, ABCB1, MRP2-4, although it has been found that CAR participates also in the regulation of glucose, lipid, and bile acids metabolisms, cellular proliferation, and cancer development (Hakkola et al., 2020; Chai et al., 2013; Kobayashi et al., 2015; Stern et al., 2022; Tolson & Wang, 2010). Its ligand-binding domain is smaller and less flexible than that of PXR and that is likely the reason

that CAR enables docking of fewer ligands (Hakkola et al., 2020). CAR is retained in cytosol as an inactive phosphorylated protein complex with heat shock protein 90 (Hsp90) and CAR cytoplasmic retention protein. Upon ligand binding, CAR is dephosphorylated by protein phosphatase 2A (PP2A), dissociated from chaperone proteins, and translocated into the cell nucleus (Hakkola et al., 2020; Stern et al., 2022). The initiation of transcription by CAR proceeds in a similar fashion as described for PXR, including heterodimerization with RXR and recruitment of coactivators like SRC proteins or PGC-1α (Kobayashi et al., 2015). Unlike PXR, ligand-independent activation of CAR by phenobarbital has been described. Phenobarbital inhibits epidermal growth factor receptor signaling which leads to dephosphorylation of CAR by PP2A and its subsequent translocation to the cell nucleus (Kobayashi et al., 2015). Interestingly, CAR and PXR share some ligands that can dock into their ligand-binding domains, and they can both recognize direct and everted repeats of nucleotides in similar XRE. They are thus able to cross-regulate the expression of several target genes, a phenomenon known as *crosstalk* (Wang et al., 2012).

AhR is a member of Class I bHLH-PAS proteins which, apart from its role as xenobiotic-sensing receptor, regulates several physiological processes, such as cell proliferation, cell adhesion and migration, and it has been implicated in the regulation of developmental processes of hematopoietic, cardiovascular, and immune systems. AhR is characteristic for its ubiquitous tissue distribution; its highest expression has been detected in the liver, placenta, lungs, heart, and pancreas. AhR is kept in cytosol in an inactive complex with two chaperone proteins Hsp90, a co-chaperone protein p23, and hepatitis B virus X-associated protein 2 (Kewley et al., 2004; Larigot et al., 2018; Mulero-Navarro & Fernandez-Salguero, 2016). The structure of AhR contains an *N*-terminal bHLH domain with DNA-binding function, two PAS domains (PAS A and PAS B) that serve for heterodimerization and ligand binding, and a transactivation domain which recruits transcriptional coactivators. After ligand binding, AhR complex translocates into the cell nucleus, dissociates from chaperone proteins, and heterodimerizes with a Class II bHLH-PAS protein, specifically with AhR nuclear translocator (ARNT). AhR/ARNT dimer binds to XRE containing sequence motif 5′-TA/TGCGTG-3′ in the promotor region of target genes and assembles a transcriptional machinery complex that encompasses several coactivator proteins with histone acetylase activity, such as SRC proteins, cAMP-responsive element-binding protein, and p300, histone methyltransferase activity, such as cofactor-associated arginine methyltransferase 1, and protein arginine methyltransferase 1, or other activities that aid in gene transcription process (Hankinson, 2005; Kewley et al., 2004; Larigot et al., 2018). Once AhR/ARNT is released from

DNA, AhR is transported back to cytosol and undergoes proteasomal degradation. Interestingly, a negative feedback mechanism has been reported for AhR, for it also initiates transcription of its repressor protein, AhR repressor (AhRR). AhRR is structurally similar to AhR except for lacking a ligand-binding PAS B domain and possessing a transrepression domain that binds corepressor proteins instead of a transactivation domain. AhRR competes with AhR for heterodimerization with ARNT. AhRR/ARNT heterodimer then binds to XRE sites of AhR, but it is transcriptionally inactive (Kewley et al., 2004; Larigot et al., 2018; Mulero-Navarro & Fernandez-Salguero, 2016). The activation of AhR signaling pathway by prescription drugs has not been frequently observed; omeprazole and primaquine would make the exception. It is largely environmental pollutants, such as PAH, halogenated aromatic hydrocarbons, or dioxins, that act as potent AhR activators (Hakkola et al., 2020; Stejskalova et al., 2011; Yoshinari et al., 2008). In addition, polyphenols quercetin and resveratrol, as well as indole-3-carbinol from cruciferous vegetables, such as broccoli and Brussels sprouts, act as activators of AhR signaling (Larigot et al., 2018). AhR is known to induce the expression of CYP1, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and ABCG2 (Tan et al., 2010; Tolson & Wang, 2010).

Last but not least, Nrf2 belongs to the cap‘n’collar subfamily of basic-region leucine zipper transcription factors, and it is a chief regulator of cytoprotective defense mechanisms against oxidative and xenobiotic stress. Nrf2 regulates the concerted transcription of several DMEs from all three phases of biotransformation, including GSTs, UGTs, SULTs, NAD(P)H:quinone oxidoreductase (NQO) 1, carbonyl reducing enzymes, or ABC transporters. Normally, Nrf2 is kept inactive in a protein complex with Keap1 (Nrf2 repressor), constantly ubiquitinated and degraded. Xenobiotics may activate the Nrf2-mediated transcription by different mechanisms: electrophiles disrupt Nrf2-Keap1 complex by directly modifying cysteine residues of Keap1, protein-protein interaction inhibitors prevent binding of Nrf2 to Kelch propeller of Keap1, and last but not least, inhibitors of glycogen synthase kinase 3 prevent phosphorylation and subsequent ubiquitination and proteasomal degradation. Upon activation, Nrf2 is translocated to the cell nucleus, binds to ARE with sequence motif 5′-TA/TGCG TGA/C-3′ as a heterodimer with members of the small musculoaponeurotic fibrosarcoma protein family, and initiates the orchestrated transcription of target genes. Interestingly, the activation of AhR also upregulates the transcriptional activity of Nrf2 (Bai et al., 2016; Robledinos-Anton et al., 2019; Tonelli et al., 2018; Zgorzynska et al., 2021).

Post-transcriptional mechanism of enzyme induction

Post-transcriptional enzyme induction of DMEs is certainly less common than transcriptional regulation of their expression. The mechanism of this type of induction can be attributed to decreased proteasomal degradation, increased mRNA stabilization, and increased protein stabilization. Among DMEs, xenobiotic-induced post-transcriptional regulation has been very well-established for CYP2E1. Substrates of CYP2E1, such as ethanol or acetone, induce its activity by protein stabilization rather than mRNA stabilization (Lu & Cederbaum, 2008). On the other hand, CYP2A6 has been found to be regulated at the level of mRNA; its stabilization is mediated by binding of heterogeneous nuclear ribonucleoprotein A1 to 3′-untranslated region of CYP2A6 mRNA (Christian et al., 2004).

Epigenetic regulation further adds to the variability of the expression of DMEs. It is directed via the activity of noncoding microRNAs (miRNA) that act as negative transcriptional regulators, along with DNA methylation proteins and histone modifying proteins. Interestingly, it has been revealed that xenobiotics can upregulate/downregulate the expression of DMEs by altering miRNA profiles (Yu et al., 2016). The treatment with rifampin demonstrated strong negative correlation between downregulated miRNAs and induced CYPs and vice versa (Ramamoorthy et al., 2013). Similarly, imatinib treatment induced the expression of ABCG2 possibly by downregulation of miR-212 and miR-328 (Turrini et al., 2012).

2.2.2 Enzyme inhibition

Inhibition of DMEs is undoubtedly a major cause of pharmacokinetic interactions. Enzyme inhibition results in diminished or completely blocked enzyme activity. The extent of inhibition is affected by both the characteristics of a substrate and an inhibitor. *Reversible enzyme inhibition* occurs as a result of a competition between a substrate and an inhibitor; binding of the inhibitor is usually weak and easily reversed. On the other hand, *irreversible enzyme inhibition* results from covalent binding of an inhibitor and sometimes inactivating the enzyme completely. Irreversible inhibition is usually long-lasting due to the need to synthesize the enzyme *de novo* (Pelkonen et al., 2008).

Reversible enzyme inhibition

Four different kinetic mechanisms of enzyme inhibition have been observed for DMEs – competitive, noncompetitive, uncompetitive, and mixed inhibition. However, unlike competitive, noncompetitive, and mixed inhibition mechanisms that are commonly observed for DMEs, uncompetitive inhibition occurs only rarely (Pelkonen et al., 2008). Competitive inhibition represents by far the most frequent and relatively straightforward mechanism. An inhibitor binds only to an unbound enzyme usually at the same active site as a substrate, and as a result, prevents the substrate from binding. With an increasing substrate concentration, however, the strength of inhibition decreases. Noncompetitive inhibitors bind to both unbound enzymes and enzyme-substrate complexes with the same binding affinity. In this case, the substrate concentration does not really affect the extent of enzyme inhibition. During uncompetitive inhibition, the inhibitor binds to already formed enzyme-substrate complex. Therefore, an increase in substrate concentration simultaneously increases the effectivity of inhibition. Mixed inhibition combines some characteristics of competitive and noncompetitive mechanisms, as the inhibitor binds directly to or near the active site of the unbound enzyme or enzyme-substrate complex (Venkatakrishnan et al., 2003; Zhang & Wong, 2005).

Irreversible enzyme inhibition

The underlying mechanism of irreversible enzyme inhibition (and for that matter also quasi-irreversible inhibition) lies in an *in situ* generation of reactive metabolic intermediates, it is thus also referred to as mechanism-based inhibition. To assess this type of inhibition, three characteristics must be met, namely NADPH-, concentration-, and time-dependency of enzyme inactivation. Mechanism-based inhibition usually occurs as a result of catalytic activity of a single or multiple CYP isoforms, and it is more likely to occur with substrates possessing tertiary amine function, acetylene function, or furan ring. Resulting reactive intermediates subsequently inactivate the enzyme via three different mechanisms – covalent binding to an amino acid residue within the active site, arylation/alkylation of the prosthetic heme moiety, or transient modification of prosthetic heme leading to cross-linking with CYP apoprotein, albeit this mechanism is deemed reversible. Some irreversible inhibitors, sometimes referred to as suicide inhibitors, can destroy the enzyme entirely and thus set the stage for a long-term inactivation due to the need to synthesize the protein *de novo* (Zhang & Wong, 2005; Zhou et al., 2005; Zhou, 2008).

2.2.3 Consequences of enzyme induction and inhibition

As discussed in **Chapter 2.2.1** and **Chapter 2.2.2**, xenobiotics can alter the activity and/or amount of DMEs by several mechanisms and thus lay the foundations for various pharmacological, toxicological, or pathophysiological outcomes. Although the investigation of drug-drug interactions is now fully integrated into the drug development, approval, and pharmacovigilance processes, evaluating potential herb-drug interactions is challenging due to the plethora of compounds and lack of data regarding their DME-modulatory activities (WHO, 2021; Yu et al., 2022a, 2022b).

The induction of DMEs may result in increased metabolism and decreased bioavailability and plasma concentrations of concomitantly used prescription drugs leading to sub-therapeutic doses or even therapy failure. This issue is of high importance especially when drugs with narrow therapeutic window (chemotherapeutics, immunosuppressants, antipsychotics, or antidepressants) or extensive first-pass metabolism are concerned (Pelkonen et al., 2008; WHO, 2021). A well-established case of herb-drug interactions with severe clinical outcomes is represented by hyperforin, an inherent constituent of *Hypericum perforatum* L. with antidepressant activity, that acts as a strong inducer of CYP3A4 and ABCB1 via PXR/CAR activation. Concomitant intake of *H. perforatum* L. during cyclosporin treatment resulted in acute transplant rejection due to decreased plasma concentration of the immunosuppressant. In addition, intermenstrual bleeding in women on oral contraceptives and even unwanted pregnancies have been documented after simultaneous administration of *H. perforatum* L. (Borrelli & Izzo, 2009; Prakash et al., 2015). Intermittent comedication with *Ginkgo biloba*, a PXR agonist, during a long-term treatment with efavirenz resulted in a breakthrough viremia in a patient otherwise virologically suppressed for over 10 years (Naccarato et al., 2012).

In the case of prodrugs and xenobiotics that undergo bioactivation, enzyme induction can, on the other hand, enhance their pharmacodynamic and toxic effects, respectively (Hakkola et al., 2020). PAH found in grilled meat, roasted coffee beans, or cigarette smoke are both perpetrators and precipitants of their increased toxicity resulting from enzyme induction. PAH are strong AhR agonists that upregulate the expression of CYP1 enzymes involved in their metabolism. However, by the activity of the very same enzymes, they undergo metabolic bioactivation to PAH-diol-epoxides that irreversibly bind to DNA and promote carcinogenic processes (Djordjevic et al., 2008; Stejskalova et al., 2011). The clinical impact and extent of enzyme induction depends on the potency of an inducer, its dose and pharmacokinetic properties, as well as duration and route of exposure. In addition, host-specific factors, such as

one's genetic makeup, diet, sex, age, or the presence of a disease, further affect the outcomes of enzyme induction. The extent is usually higher in individuals with lower baseline enzyme activity. In addition, the effect of enzyme induction waxes and wanes with a time delay due to the need to synthesize DMEs *de novo* (Hakkola et al., 2020; Pelkonen et al., 2008).

The inhibition of DMEs interferes with the pharmacokinetics of co-administered drugs in the opposite way. As a result, drug clearance is decreased leading to high plasma levels and bioaccumulation of active compounds with potential manifestation of their adverse/toxic effects. In addition, bioactivation of prodrugs can be decreased resulting in sub-therapeutic plasma concentrations of active metabolites. Several factors including the characteristics of an inhibitor as well as co-administered drugs contribute to the severity of clinical outcomes of enzyme inhibition. Irreversible (mechanism-based) enzyme inhibitors are in general of higher risk of provoking herb-drug interactions as *de novo* synthesis of affected enzymes is required. In the same vein, drugs with narrow therapeutic window as well as those with one major detoxification/bioactivation pathway are more likely to be affected by enzyme inhibition (Fasinu et al., 2012; Hakkola et al., 2020; Pelkonen et al., 2008). Many constituents of herbal products have shown an inhibitory activity towards DMEs (Kennedy & Seely, 2010; Liu et al., 2019). Among them, furanocoumarins found in grapefruit juice are well-established and potent mechanism-based inhibitors of hepatic and intestinal CYP3A4 that also interfere with the activity of uptake and efflux transporters and thus largely affect the metabolism and excretion of concomitantly administered drugs (Bailey et al., 2007; Cederbaum, 2015; He et al., 1998; Tassaneeyakul et al., 2000). Co-administration of polyphenolic compounds baicalein and psoralidin during irinotecan treatment heightened the gastrointestinal toxicity of the chemotherapeutic due to the inhibition of UGT1A1 (Liu et al., 2019).

However, the inhibition of DMEs implicated in the bioactivation of carcinogens may also show positive biological effects, mainly in the prevention of the development of malignancies. Natural compounds with antitumor activity are often inhibitors of CYP1 enzymes. In particular, CYP1B1 is regarded as an interesting protein target as it is involved not only in the metabolic bioactivation of xenobiotics, but also endogenous compounds such as estrogen. In addition, its expression is induced by pro-inflammatory cytokines, further contributing to the tumor progression (Badal & Delgoda, 2014; Baer-Dubowska & Szaefer, 2013; D'Uva et al., 2018). Similarly, the inhibition of CYP2A13 that bioactivates NNK found in cigarette smoke might act as a chemopreventive mechanism against lung cancer (Vrzal, 2021).

2.3 Natural compounds as modulators of drug-metabolizing enzymes

2.3.1 Sesquiterpenes

Sesquiterpenes are secondary metabolites produced mainly by higher plants. Structurally, they encompass a group of 15-carbon terpenoids built from three isoprene units. Sesquiterpenes form an inherent part of human diet; in addition, they demonstrated various health-promoting effects featuring their anti-inflammatory, antitumor, or antioxidant activity. For that reason, they are also frequently included in popular herbal products and dietary supplements (Bartikova et al., 2014). Due to their valuable physicochemical properties, sesquiterpenes are of use also in cosmetic, pharmaceutical, and food industry as fragrances, flavoring and antimicrobial agents, or as enhancers of transdermal permeation (Caputi & Aprea, 2011; Kontogiannidou et al., 2017; Williams & Barry, 2004). In this dissertation, three acyclic sesquiterpenes (farnesol, *cis*-nerolidol, *trans*-nerolidol), three cyclic sesquiterpenes (α-humulene, β-caryophyllene, caryophyllene oxide), and one sesquiterpene lactone (helenalin) were studied (**Figure 2**) and will be further discussed mainly with regard to their previously reported DME-modulatory activity.

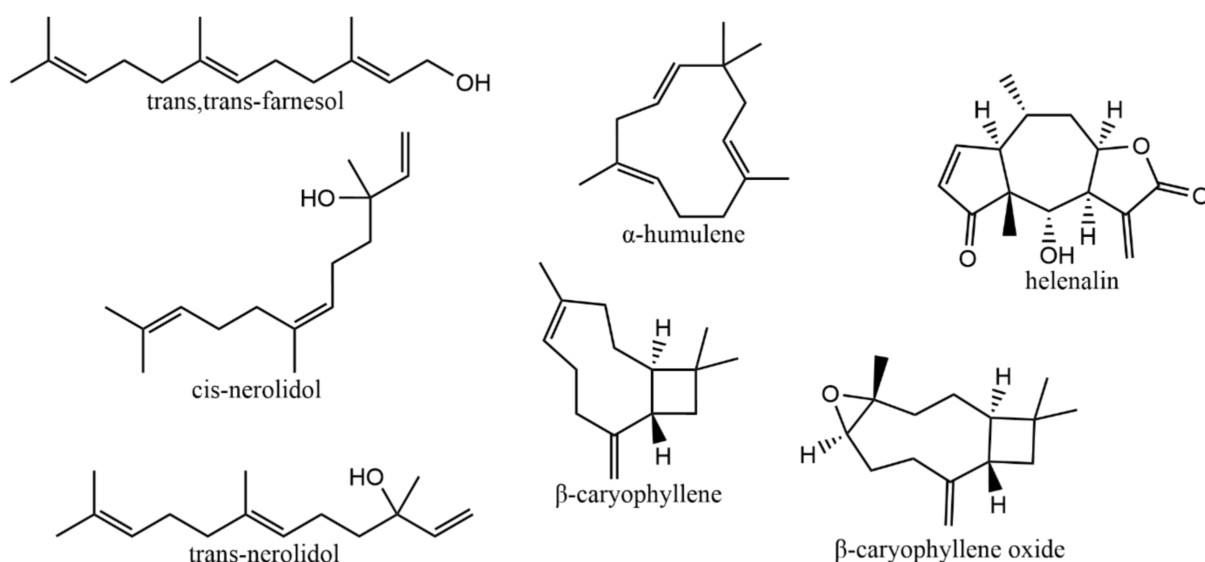

Figure 2 Chemical structures of the studied sesquiterpenes.

Acyclic sesquiterpenes

Farnesol (*trans,trans*-farnesol) exhibits excellent anti-inflammatory and anti-cancer activity against several cancer types both *in vitro* and *in vivo* (Jung et al., 2018). In addition, it has been recently studied as a lipid-lowering agent (Pant et al., 2019). Concerning its DME-modulatory activity, farnesol increased the mRNA expression of CYP2B6 and CYP3A4 and decreased the expression of CYP2E1 in differentiated HepaRG cells (Pant et al., 2019). The increase in CYP2B6 expression might be a result of CAR activation, as farnesol increased the CYP2B6-PBREM/XREM reporter activity in CAR-knockout mouse hepatocytes transfected with either human or mouse CAR (Rondini et al., 2016). Farnesol acted as a very weak inducer of CYP2C in human tongue squamous cells (CAL 27) and HepG2 cells (Yang & Raner, 2005). On the other hand, it significantly inhibited the activity of CYP1A2 and CYP2B/3A in human and rat liver microsomes as determined by ethoxyresorufin-*O*-deethylase (EROD) and benzoxyresorufin-*O*-dealkylase (BROD) activity assays, respectively, but it had no effect on carbonyl reducing enzymes or phase II DMEs. However, when different CYP substrates were used, no inhibition was observed (Spicakova et al., 2017). A weak inhibition of human recombinant UGT2B7 by farnesol has also been reported (Staines et al., 2004). After repeated administration of a non-toxic dose of farnesol to rats, the activity of all main CYPs as well as UGT, glutathione reductase and NQO increased in the liver (Horn et al., 2005)

Nerolidol exists in two stereoisomeric forms as *cis*-nerolidol and *trans*-nerolidol that both exhibit neuroprotective and anti-cancer activity *in vitro* and *in vivo*. Furthermore, it is active against a wide range of parasites and acts as a potent transdermal penetration enhancer (Chan et al., 2016). Similarly to farnesol, both *cis*-nerolidol and *trans*-nerolidol inhibited the activity of CYP1A and CYP2B/3A in human and rat liver microsomes when evaluated with EROD and BROD assays, respectively. Although a weak inhibition of CYP3A4/5 and CYP2C19 was observed in human liver microsomes when other CYP substrates were used. In addition, neither of the tested compounds affected the activity of carbonyl reducing enzymes or phase II DMEs (Spicakova et al., 2017). In mice *in vivo*, a single administration of *trans*-nerolidol increased the activity and mRNA expression of hepatic and intestinal CYP2B, CYP2C, and CYP3A. *trans*-Nerolidol further increased the activity of hepatic AKR1C and SULT and decreased the activity of intestinal NQO1 (Lnenickova et al., 2018).

Cyclic sesquiterpenes

α-Humulene demonstrated anti-inflammatory and antitumor activity both *in vivo* and *in vitro*. Apart from that, α-humulene stands as an antimicrobial agent (de Lacerda Leite et al., 2021). However, there is only limited information regarding its DME-modulating activity in humans. Nevertheless, α-humulene inhibited the activity of CYP2B/3A in human and rat liver microsomes as estimated by BROD assay; in addition, the extent of inhibition in the former was comparable to that of ketoconazole. However, its inhibitory activity was not confirmed with other CYP2B/3A substrates, nor was it detected for other phase I or phase II DMEs even at a relatively high α-humulene concentration (Nguyen et al., 2017).

β-Caryophyllene is an interesting bicyclic sesquiterpene that acts as a selective agonist of cannabinoid receptor 2. Activation of the cannabinoid receptor stands behind its analgesic, immunomodulatory, antioxidant, and antitumor activity. β-Caryophyllene has been also found to regulate glucose and lipid homeostasis as well as to attenuate withdrawal symptoms and reduce the administered dose in alcohol/nicotine use disorders. Despite the fact that several herbal preparations rich in β-caryophyllene have been approved for internal use (Hashiesh et al., 2021), there is only one study that investigated the modulatory effect of β-caryophyllene on DMEs. In the study, β-caryophyllene was found to inhibit the activity of CYP3A/2B in human and rat liver microsomes comparably to ketoconazole when assessed with BROD assay, but showed no effect when other CYP3A4 of CYP2B6 substrates were used. β-Caryophyllene had also no effect on carbonyl reducing enzymes or tested phase II DMEs (Nguyen et al., 2017).

β-Caryophyllene oxide does not bind to the cannabinoid receptor as does β-caryophyllene, and thus lacks the analgesic activity. However, β-caryophyllene oxide is known to inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and signal transducer and activator of transcription 3 (STAT3) signaling pathways involved in cell proliferation, survival, and invasion. In addition, it boosts the efficacy of commonly used antineoplastic agents *in vitro* (Fidyt et al., 2016). Regarding its DME-modulatory activity, β-caryophyllene oxide inhibited the activity of CYP2B/3A in human and rat hepatic subcellular fractions in a similar manner to β-caryophyllene but with slightly more potency. In addition, β-caryophyllene oxide also inhibited midazolam hydroxylation catalyzed by CYP3A4/5, but it did no inhibit other phase I or phase II enzymes (Nguyen et al., 2017). When a single dose of β-caryophyllene oxide was administrated to mice, increased activities and mRNA levels of hepatic and intestinal CYP2B, CYP3A, and CYP2C, and decreased activity of intestinal NQO1 were detected (Lnenickova et al., 2018).

Sesquiterpene lactones

Helenalin is a potent anti-inflammatory, antiprotozoal, and antitumor agent. Its anti-inflammatory activity is mediated by the selective inhibition of NF-κB (Drogosz & Janecka, 2019). Furthermore, the investigation of its hepatoprotective activity against liver injury and fibrosis is underway (Li et al., 2019; Lin et al., 2014; Xiong et al., 2022). However, helenalin also showed a strong CYP inhibitory activity in rodents. It decreased the hepatic microsomal CYP content and acted as an inhibitor of aminopyrine demethylase, aniline hydroxylase, and 7-ethoxyresorufin deethylase in mice *in vitro* as well as *in vivo*. Its inhibitory effect was more pronounced in the presence of NADPH (Chapman et al., 1989, 1991; Chapman et al., 1988). In addition, intragastric administration of helenalin to rats decreased their hepatic CYP content and the activities of aniline hydroxylase, aminopyrine demethylase, NADPH-CYP reductase, and GST (Jodynis-Liebert et al., 2000). So far, the inhibitory activity of helenalin has not been tested in humans.

2.3.2 *Prenylflavonoids*

Prenylflavonoids are important dietary polyphenols possessing a basic diphenyl propane skeleton with an attached prenyl moiety. Prenylation enhances their biological activity and tissue bioaccumulation compared to their unprenylated counterparts (Mukai, 2018; Mukai et al., 2013). *Humulus lupulus* L. (hops) is a rich source of bioactive prenylflavonoids featuring in particular xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin, commonly referred to as hop prenylflavonoids (**Figure 3**). Female inflorescences of *H. lupulus* L. are used as a raw material in brewing industry and are responsible for the characteristic bitter taste of beer (Stevens & Page, 2004; Zanoli & Zavatti, 2008). Additionally, extracts from hops exert estrogenic activity that aids in relieving postmenopausal symptoms (Bolton et al., 2019).

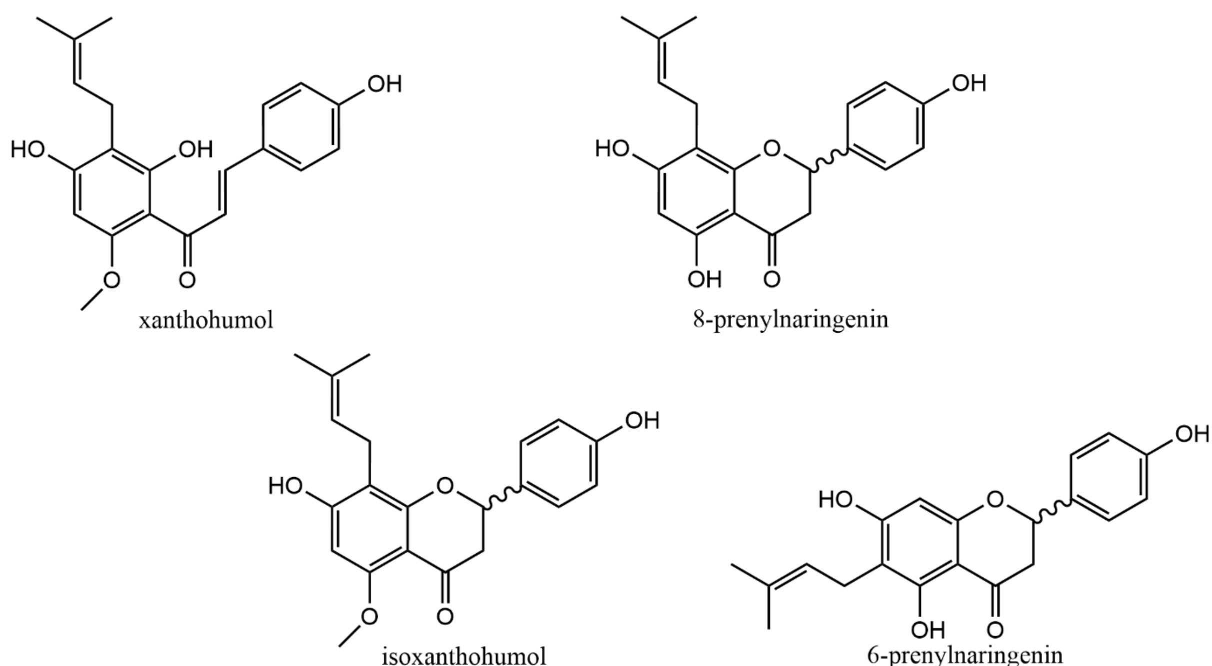

Figure 3 Chemical structures of the studied hop prenylflavonoids.

Similarly, individual hop prenylflavonoids *per se* possess a multitude of health-beneficial activities. Xanthohumol acts as a chemopreventive and anti-inflammatory agent due to the inhibition of NF-κB and STAT3 signaling pathways, which inherently leads to the suppression of cell proliferation and induction of apoptosis (Albini et al., 2006; Dokduang et al., 2016). In addition, it activates both intrinsic and extrinsic pathways of apoptosis (Pan et al., 2005). The activation of AMPK signaling plays a role in its antiangiogenic as well as its anti-obesity and

antiglycemic activity (Bolton et al., 2019). 8-Prenylnaringenin is a very potent agonist of estrogen receptor (and the most potent phytoestrogen for that matter), and as such it is the chief constituent of hop-containing herbal products responsible for alleviating the negative symptoms accompanying menopause (Overk et al., 2005; Stulikova et al., 2018). Its stereoisomer, 6-prenylnaringenin, exhibits a chemopreventive effect against breast cancer through direct modulation of estrogen metabolism enhancing its detoxification rather than its genotoxic metabolic pathway (Wang et al., 2016; Ziegler et al., 2015). Lastly, isoxanthohumol exhibits promising anti-cancer and antiviral activity, but it is simultaneously the least studied prenylflavonoid (Zolnierczyk et al., 2015).

Hop prenylflavonoids have been found to be positive and also negative modulators of several transcription factors and are thereby involved in the regulation of their downstream targets. Xanthohumol activated Nrf2 signaling and target gene transcription via directly alkylating Keap1 in Keap1-Nrf2 protein complex. In rats *in vivo*, Nrf2 activation led to the increase in hepatic NQO1 and GST activity; in normal THLE-2 hepatocytes, such activation yielded increased mRNA and protein expression of GSTP, GSTT, NQO1, and heme-oxygenase 1 (Dietz et al., 2013; Krajka-Kuzniak et al., 2013). Contrarily, xanthohumol acted as a negative regulator of CAR in rats *in vivo* as it diminished both its mRNA and protein levels. In line with this observation was the downregulation of CYP2B15, SULT1A1, and UGT1A1, which expression is also regulated by CAR (Radovic et al., 2010). Furthermore, 6-prenylnaringenin and 8-prenylnaringenin activated the transcriptional activity of AhR in HepG2 cells and might therefore be regarded as potential AhR ligands (Wang et al., 2016). Besides transcriptional regulation, hop prenylflavonoids exhibited direct inhibitory activity towards several phase I DMEs, although they each had different enzyme preferences. All four prenylflavonoids were potent inhibitors of human CYP1B1, CYP2C8, and CYP2C9. Xanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin were also potent inhibitors of CYP1A1 and weak inhibitors of CYP2B6. Last but not least, only 8-prenylnaringenin and 6-prenylnaringenin inhibited the activity of CYP1A2 and CYP2C19. Neither of the hop prenylflavonoids exhibited affinity towards CYP3A4 or CYP2E1 (Henderson et al., 2000; Yuan et al., 2014). Similar inhibitory activity and differing enzyme preferences were also reported for carbonyl reducing enzymes. 8-Prenylnaringenin, isoxanthohumol, and xanthohumol reduced the activity of CBR1, yet only the first two prenylflavonoids inhibited also the activities of AKR1B1 and AKR1B10 (Seliger et al., 2019; Seliger et al., 2018).

3 AIMS OF THE DISSERTATION

This dissertation aimed to bring new pieces of information regarding the ability of selected sesquiterpenes and prenylflavonoids to modulate the activity and expression of phase I and phase II DMEs in hepatic and intestinal *in vitro* models as well as to investigate their biotransformation pathways.

Specific aims of this dissertation included:

i. determination of the ability of six sesquiterpenes – farnesol, *cis*-nerolidol, *trans*-nerolidol, α-humulene, β-caryophyllene, and caryophyllene oxide, to act as agonists of xenobiotic receptors and to modulate gene and protein expression of phase I DMEs in human precision-cut liver slices;
ii. investigation of the NADPH-dependent metabolism of a sesquiterpene lactone helenalin and its ability to alter the activity of CYP enzymes using hepatic fractions and human recombinant CYP proteins;
iii. investigation of the effect of four prenylflavonoids – xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin, on the activity and gene expression of phase II DMEs in proliferating and differentiated CaCo-2 cells.

4 PUBLICATIONS INCLUDED IN THE DISSERTATION WITH CANDIDATE'S CONTRIBUTION

This dissertation is composed in the form of an annotated set of peer-reviewed articles published in impacted international scientific journals. Information about the journal impact factor (IF) and quartile based on journal IF (Q) in the year of publishing is indicated for each publication.

4.1 Publication I

Šadibolová, M., Zárybnický, T., Smutný, T., Pávek, P., Šubrt, Z., Matoušková, P., Skálová, L., & Boušová, I. (2019). Sesquiterpenes Are Agonists of the Pregnane X Receptor but Do Not Induce the Expression of Phase I Drug-Metabolizing Enzymes in the Human Liver. *International Journal of Molecular Sciences*, *20*(18), 4562. **(IF_{2019} = 4.556, Q1)**

Candidate's contribution

- performance of western blotting experiments
- data analysis, data interpretation, and data visualization
- participation in the writing of the manuscript

4.2 Publication II

Šadibolová, M., Juvonen, R. O., Auriola, S., & Boušová, I. (2022). In vitro metabolism of helenalin and its inhibitory effect on human cytochrome P450 activity. *Archives of Toxicology*, *96*(3), 793–808. **(IF_{2022} = 6.1, Q1 - first decile**)

Candidate's contribution

- study design and performance of all experiments
- data analysis, data interpretation, and data visualization
- writing of the manuscript and preparation for submission
- revision of the manuscript

4.3 Publication III

Lněničková, K., **<u>Šadibolová, M.</u>**, Matoušková, P., Szotáková, B., Skálová, L., & Boušová, I. (2020). The modulation of phase II drug-metabolizing enzymes in proliferating and differentiated CaCo-2 cells by hop-derived prenylflavonoids. *Nutrients*, *12*(7), 2138. **(IF_{2020} = 5.719, Q1)**

Candidate's contribution

- performance of the experiments
- data analysis, data interpretation

5 RESULTS

5.1 Sesquiterpenes and hepatic phase I drug-metabolizing enzymes

5.1.1 Modulation of mRNA and protein expression

Publication I: Šadibolová, M., Zárybnický, T., Smutný, T., Pávek, P., Šubrt, Z., Matoušková, P., Skálová, L., & Boušová, I. (2019). Sesquiterpenes Are Agonists of the Pregnane X Receptor but Do Not Induce the Expression of Phase I Drug-Metabolizing Enzymes in the Human Liver. *International Journal of Molecular Sciences*, *20*(18), 4562.

Herbal products containing sesquiterpenes have a long record of use in the treatment of human diseases (Bartikova et al., 2014). On the other hand, their safety assessment or evaluation of possible herb-drug interactions in humans have been neglected so far. In our first study, we therefore screened three members of acyclic sesquiterpenes – farnesol, *cis*-nerolidol, and *trans*-nerolidol, and three members of cyclic sesquiterpenes – α-humulene, β-caryophyllene, and β-caryophyllene oxide, for their modulatory effect on hepatic phase I DMEs.

All tested sesquiterpenes elevated the activity of PXR (but not that of AhR) in a concentration-dependent manner as assessed by luciferase reporter gene assay. Amongst them, *cis*-nerolidol and *trans*-nerolidol were the most potent activators of PXR increasing its activity 2.4× and 2.1×, respectively, at 10 µM concentration, and they further elevated its activity 5.5× and 4.3×, respectively, at 30 µM concentration. For comparison, 10 µM rifampicin, a reference PXR agonist, elevated PXR activity approximately 8-fold; thus, a mild inducing activity was concluded for sesquiterpenes.

Therefore, we further proceeded with testing their modulatory effect at 10 µM concentration on the mRNA and protein expression of the two downstream PXR targets – CYP3A4 and CYP2C using human precision-cut liver slices. We also tested their effect on the main hepatic carbonyl reducing enzymes – AKR1C and CBR1, which are indeed the downstream targets of Nrf2 (Ebert et al., 2016; Penning et al., 2019), but Nrf2-activation effect has been already observed for other structurally similar sesquiterpenes (Nakamura et al., 2004). In our study, neither of the tested compounds provoked noticeable changes in the expression of selected phase I DMEs. Moreover, the few weak changes observed were not consistent in between individual precision-cut liver slices obtained from different tissue donors, but rather affected

by interindividual variability. It can be thus tentatively concluded that the studied sesquiterpenes would not be causative of significant herb-drug interactions.

5.1.2 Biotransformation and inhibitory activity toward cytochromes P450

Publication II: **Šadibolová, M.**, Juvonen, R. O., Auriola, S., & Boušová, I. (2022). In vitro metabolism of helenalin and its inhibitory effect on human cytochrome P450 activity. *Archives of Toxicology*, *96*(3), 793–808.

This study aimed to comprehensively elucidate the biotransformation of a sesquiterpene lactone helenalin as well as to further research its previously reported inhibitory activity on CYPs. Firstly, NADPH-dependent metabolism of 5 µM and 100 µM helenalin was studied in human and rat liver microsomes using UHPLC-Q-Exactive-MS/MS. Helenalin was oxidized into five metabolites (assigned M1-M5) in both species; in addition, two reduced metabolites (M6 and M7) were found only in human subcellular fractions. Interestingly, no glucuronide or sulfate conjugates were detected, and conjugation with glutathione was rather spontaneous. The biotransformation pathway of helenalin is depicted in **Figure 4**.

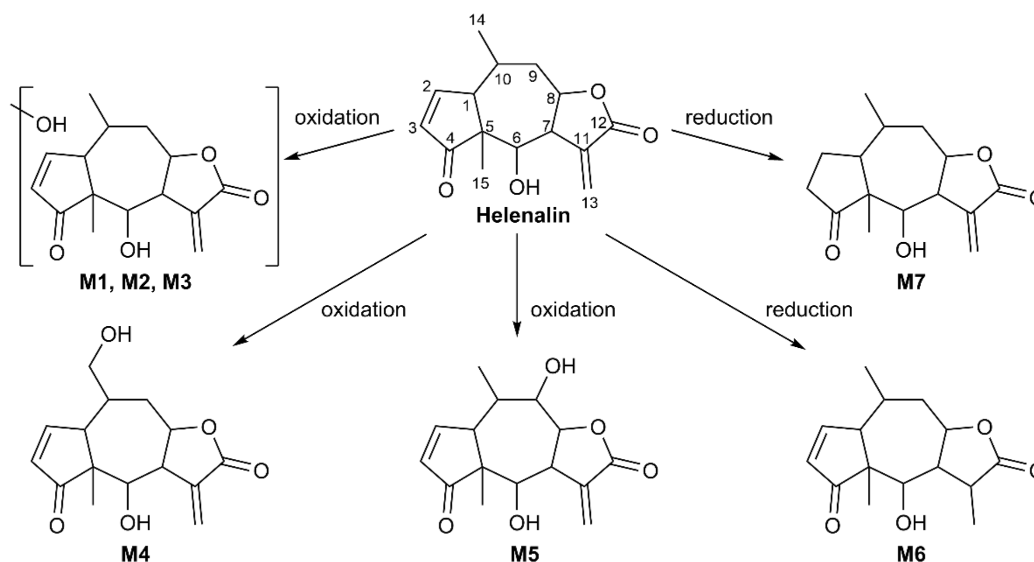

Figure 4 NADPH-dependent biotransformation of helenalin and proposed molecular structures of its metabolites.

Overall, the NADPH-dependent metabolism of helenalin differed between the two studied species in both studied aspects – in the total profile of produced metabolites as well as in the pharmacokinetic parameters of biotransformation. Firstly, 14-hydroxyhelenalin (M4) was the major metabolite found in human liver microsomes, whereas 9-hydroxyhelenalin (M5) was the predominant metabolite in rat liver microsomes. In addition, the reduction of helenalin only took place in human subcellular fractions. Secondly, the NADPH-dependent oxidation of helenalin into three major metabolites (M3, M4, and M5) was more efficient in rat liver microsomes as the sum of all values of intrinsic clearance was about three times higher compared to the values calculated for human liver microsomes.

Subsequently, a detailed characterization of individual human CYP isoforms contributing to the oxidative metabolism of helenalin was performed. Surprisingly, the extrahepatic CYP2A13 was the most efficient CYP isoform with the highest values of intrinsic clearance and the highest affinity for the formation of metabolites M3 and M4. It was followed by hepatic CYP3A5, which activity yielded the three main metabolites, and CYP3A4 that only catalyzed the formation of M4. Surprisingly, CYP3A5 was 1.5× more efficient in oxidating helenalin into M4 than CY3A4 and demonstrated different regioselectivity compared to CYP3A4. CYP2B6 metabolized helenalin into M5 and it was the only isoform catalyzing the formation of the two minor metabolites, M1 and M2. However, the formation of metabolite M5 by CYP2B6 did not follow Michaelis-Menten kinetics.

Last but not least, we confirmed some inhibitory activity towards human CYPs, as helenalin acted as a competitive inhibitor of CYP3A4 with a half-maximal inhibitory concentration (IC_{50}) of 18.7 μM, a weak inhibitor of CYP3A5 with IC_{50} of 62.6 μM, and a selective mechanism-based inhibitor of CYP2A13 (IC_{50} = 1.1 μM) with inhibitor concentration required for a half-maximal rate of inactivation (K_I) of 6.7 μM and the inactivation rate constant at an infinite concentration of inhibitor (k_{inact}) of 0.58 ln(%)/min. These results tentatively suggest that helenalin might have some effect on concomitantly used medication.

5.2 Prenylflavonoids and intestinal phase II drug-metabolizing enzymes

5.2.1 Modulation of enzyme activity and mRNA expression

Publication III: Lněničková, K., **Šadibolová, M.**, Matoušková, P., Szotáková, B., Skálová, L., & Boušová, I. (2020). The modulation of phase II drug-metabolizing enzymes in proliferating and differentiated CaCo-2 cells by hop-derived prenylflavonoids. *Nutrients*, *12*(7), 2138.

The aim of the last study was to screen a group of prenylflavonoids for their modulatory activity of the main intestinal phase II DMEs using CaCo-2 cell line as a well-established *in vitro* model of enterocyte-like cells. We also compared their effect in proliferating versus differentiated CaCo-2 cells.

First of all, we determined the cytotoxicity of selected prenylflavonoids in both proliferating and differentiated CaCo-2 cells. The cytotoxic effect of prenylflavonoids was time- and concentration-dependent, and much more pronounced in proliferating CaCo-2 cells. Xanthohumol was found to exhibit the strongest antiproliferative effect on proliferating CaCo-2 cells with IC_{50} of 8.5 μM and 6.2 μM after 24- and 72-hour incubation, respectively.

Based on the cytotoxicity results, prenylflavonoids at the final concentration of 1 μM were further used. Overall, DMEs in differentiated CaCo-2 cells were more susceptible to the modulatory effects of prenylflavonoids. In differentiated cells, all prenylflavonoids decreased the activity of SULT and increased the activity of COMT following 72-hour incubation without exerting any effect on their gene expression. Furthermore, 6-prenylnaringenin and 8-prenylnaringenin also increased the total activity of GST and such trend was also observed for the other two prenylflavonoids, though it was not significant. Despite the elevated activity of total GST, all prenylflavonoids significantly decreased the mRNA expression of GSTA1/2. The inhibition of GSTA1/2 gene expression was generally observed after 24-hour incubation and disappeared following longer incubation time after which the above-mentioned increase in the enzyme activity was observed. Similarly, a significant inhibition of GSTA1/2 gene expression was observed in proliferating CaCo-2 cells; however, it was detected only after 72-hour incubation with no accompanying change in the overall GST activity. With regard to UGT1A6, there was a general trend towards increasing its activity in differentiated CaCo-2 cells upon prenylflavonoid treatment; however, only isoxanthohumol and 6-prenylnaringenin provoked a significant increase. Based on the presented results, the possibility of herb-drug interaction cannot be excluded, though further research is needed to obtain clear-cut results.

6 DISCUSSION

Concomitant use of herbal products with prescription medicines are the foreground of unintended and potentially health-endangering herb-drug interactions. In addition, thorough assessment and detailed understanding of the mechanisms of herb-drug interactions are challenging due to the complexity of herbal products, plethora of inherently present constituents, or synergistic/antagonistic effects of multiple constituents (WHO, 2021). The effort to identify potential perpetrators and the underlying mechanism of interactions is, therefore, of high importance. With that in mind, this dissertation endeavored to bring new pieces of information about selected constituents of herbal products under-researched with regard to their ability to provoke herb-drug interactions caused by the modulation of DMEs.

Our scientific research focused firstly on a group of sesquiterpenes due to their wide range of applications in pharmaceutical, cosmetic, and food industry, as well as their well-established health-beneficial activities (Bartikova et al., 2014). Following oral administration, the maximal plasma concentration of selected sesquiterpenes varied from units to lower tens of micromolar range depending on the compound and administered dose; in addition, their high affinity towards hepatic tissue has been observed (Hou et al., 2021; Chaves et al., 2008; Liu et al., 2013; Saito et al., 2015). We therefore tested their capability to modulate the expression of the main phase I DMEs. In our study, all chosen sesquiterpenes elevated the transcriptional activity of PXR; however, the inducing effect was not translated into clinically significant changes in the mRNA or protein expression of its downstream targets CYP3A4 and CYP2C. In addition, no significant changes were detected for the most abundant hepatic carbonyl reductases, AKR1C and CBR1. Contrarily, significant increase in the mRNA expression and enzyme activity of hepatic CYP3A4 and CYP2C were detected after 24 hours following oral administration of β-caryophyllene and *trans*-nerolidol (50 mg/kg) to mice (Lnenickova et al., 2018). The extrapolation of results between different species and different experimental approaches (*in vivo* versus *in vitro*) is challenging as many different factors affect the outcomes. Such factors include but are not limited to species-specific activation of transcription factors as a result of structural changes of their ligand-binding domains (Tolson & Wang, 2010) or the extent of biotransformation leading to inactive metabolites (Martignoni et al., 2006). As precision-cut liver slices represent a widely accepted model for induction/inhibition studies (de Graaf et al., 2007; de Graaf et al., 2010; Elferink et al., 2011), it might be concluded that neither of the tested

sesquiterpenes would be likely to affect the pharmacokinetics of concomitantly used drugs, although further studies are needed to confirm their safety with regard to herb-drug interactions.

Sesquiterpene lactones comprise a group of pharmacologically interesting compounds with a broad spectrum of biological effects featuring in particular anti-inflammatory and antitumor activity. In addition, with the advent of artemisinin – a sesquiterpene lactone officially approved for the treatment of malaria caused by *Plasmodium falciparum*, the research interest in sesquiterpene lactones have risen (Moujir et al., 2020; White, 2008). Therefore, we further focused our attention on one member of this group – helenalin as there is ample evidence about its therapeutic potential but a lack of it with regard to its metabolism or pharmacokinetic properties. The knowledge about biotransformation pathways of drugs and other xenobiotics provides an important tool for drug discovery and safety assessment. Identified metabolites can be synthesized and subjected to further screening, which might lead into the discovery of derivatives with improved activity, toxicity, and pharmacokinetic profiles. Detection of glutathione conjugates or bioactivated metabolites might reveal plausible perpetrators of adverse effects and idiosyncrasies (Ravindran et al., 2012; Shanu-Wilson et al., 2020). Identification of selective contribution of highly polymorphic enzymes often results in the withdrawal of a drug candidate from further testing (Ingelman-Sundberg, 2001). In toxicology, specific metabolites can be exploited as biomarkers of exposure (Lison, 1999).

We characterized two main biotransformation pathways of helenalin in humans – oxidation and reduction. With regard to the former, several CYP isoforms, especially CYP2A13, CYP3A4, CYP3A5, and CYP2B6, contributed to the oxidative metabolism. Surprisingly, the extrahepatic CYP2A13 was the most efficient isoform exhibiting the highest values of intrinsic clearance and the highest affinity. In spite of the overlapping substrate specificity between CYP2A13 and CYP2A6 (Zanger & Schwab, 2013), the contribution of the latter was insignificant. High efficiency of CYP2A13 has also been reported towards several coumarins (Fayyaz et al., 2018; Juvonen et al., 2019; Zanger & Schwab, 2013), suggesting its preference for low molecular weight aromatic compounds with electrophilic moieties. On the other hand, the catalytic activity of CYP2A13 possibly yielded a reactive metabolite of helenalin, as a selective and relatively potent mechanism-based inhibition of this isoform was observed. Although we were able to identify the sites of hydroxylation of the two major metabolites, the structure of helenalin contains several features that might allow for the formation of reactive metabolites, such as epoxides. CYP2A13 plays a leading role in the bioactivation of a tobacco procarcinogen NNK and subsequent development of related malignancies. Individuals with impaired activity of CYP2A13 face a lower risk of tumor manifestation; targeted

pharmacological inactivation might therefore represent a possible means of chemoprevention (Boonruang et al., 2017).

The acquired *in vitro* results nonetheless imply that with regard to the hepatic metabolism, CYP3A4 and CYP3A5 (and to a limited extent CYP2B6) would play the leading roles. In general, CYP3A5 usually exhibits reduced catalytic activity for xenobiotic or endogenous substrates in comparison with CYP3A4 (Niwa et al., 2019; Niwa et al., 2020; Williams et al., 2002); however, its activity towards helenalin was surprisingly higher compared to that of CYP3A4. In addition, different regioselectivity of these two CYP3A isoforms was noticed with CYP3A5 catalyzing the oxidation at three different sites. Only a limited regioselectivity differences were reported for the two main CYP3A isoforms. The expression of CYP3A5 is furthermore highly affected by genetic polymorphism (Zanger & Schwab, 2013), thus high interindividual and interethnic differences in the biotransformation of helenalin can be presumed. Given that helenalin competitively inhibited mainly the activity for CYP3A4, some pharmacokinetic interactions might be plausible.

Following up on elucidating the modulatory effects of natural compounds on DMEs, we tested a group of prenylflavonoids inherently present in *Humulus lupulus* L. Herbal products containing extracts from *H. lupulus* L. are commonly used by women to relieve from menopausal symptoms (Bolton et al., 2019). In addition, hops are largely used in the brewing industry, and therefore the dietary exposure to prenylflavonoids comes predominantly from beer consumption (Stevens & Page, 2004). It has been already established that the absorption of prenylflavonoids is quite low and that they undergo extensive conjugation metabolism. In addition, they are likely to diffuse through the cell membranes and accumulate in enterocytes (Mukai, 2018; Mukai et al., 2013; Pang et al., 2007). We therefore intentionally screened for their modulatory activity on conjugating DMEs found in the intestinal wall using differentiated and proliferating CaCo-2 cells as a well-established *in vitro* model.

Differentiated CaCo-2 cells lose the tumorigenic phenotype and possess small intestinal enterocyte-like features, they are thus employed as a surrogate of intestinal tissue (Stierum et al., 2003). The treatment of differentiated CaCo-2 cells with hop prenylflavonoids resulted in an increase in the enzyme activities of GST and COMT and decrease in the enzyme activity of SULT. Elevation of hepatic GST enzyme activity as well as reduced expression of SULT1A1 following the treatment with xanthohumol has been also reported in rats (Dietz et al., 2013; Radovic et al., 2010). Given the protective roles that conjugation enzymes play in tackling potentially harmful and reactive compounds (Jancova et al., 2010), the upregulation of the enzyme activities of GST and COMT may be deemed beneficial. In addition, pretreatment with

prenylflavonoids can alleviate the damage caused by oxidative stress and reactive oxygen species via the very upregulation of the detoxification enzymes (Dietz et al., 2013). On the other hand, any noticeable change in the catalytic activities of the major conjugating enzymes in the intestines might seriously affect the first-pass elimination of orally administered drugs, and subsequently affect their bioavailability, excretion, and plasma concentration. Thus, caution needs to be taken when dietary products containing hop prenylflavonoids are administered together with prescription drugs; however, further investigation is needed.

7 CONCLUSIONS

This dissertation focused on the interaction of plant secondary metabolites from the class of sesquiterpenes and prenylflavonoids with the hepatic and intestinal battery of biotransformation enzymes. The original results of the dissertation can be summarized in the following points:

i. Three acyclic sesquiterpenes – farnesol, *cis*-nerolidol, *trans*-nerolidol, and three cyclic sesquiterpenes – α-humulene, β-caryophyllene, β-caryophyllene oxide, were screened for their modulatory effects on the main hepatic CYPs and carbonyl reducing enzymes. Despite activating PXR-mediated gene transcription, neither of the tested sesquiterpenes provoked any clinically significant changes in the mRNA or protein expression of the studied enzymes in human precision-cut liver slices suggesting low risk of potential herb-drug interactions.

ii. NADPH-dependent biotransformation of a sesquiterpene lactone helenalin was characterized in detail using human hepatic subcellular fractions and recombinant CYPs. Helenalin underwent phase I oxidation and reduction, and no phase II conjugates were detected. The oxidation of helenalin was catalyzed by CYP2A13, CYP3A4, CYP3A5, and CYP2B6. Interestingly, CYP2A13 metabolized helenalin the most efficiently, albeit it was in turn inactivated in a mechanism-based fashion. In addition, helenalin acted as a competitive inhibitor of CYP3A4. Interspecies comparison between human and rat samples revealed differences in the overall profiles of detected metabolites as well as in the rate of metabolism.

iii. Four prenylflavonoids from hops – xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin, were tested for their modulatory effects on the main intestinal conjugation enzymes using proliferating and differentiated CaCo-2 cells. Collectively, the modulatory effect was much more pronounced in differentiated CaCo-2 cells. Therein, prenylflavonoids increased the overall activity of GST and COMT, and decreased the activity of SULT. Moreover, isoxanthohumol and 6-prenylnaringenin also elevated the mRNA expression of UGT1A6. All in all, changes in the activity and/or mRNA expression of the main conjugation enzymes mediated via prenylflavonoids pose some risk of manifesting herb-drug interactions and further research should be undertaken.

8 DISSEMINATION OF RESEARCH FINDINGS

8.1 Presentations related to the topic of the dissertation

Oral presentations:

- **Šadibolová, M.**, Zárybnický, T., Trnčáková, V., Deingruberová, K., Ambrož, M., Šubrt, Z., Skálová, L., Boušová, I. (Jan 2019). The effect of sesquiterpenes on enzymes involved in detoxification processes in humans. *9th Postgraduate and 7th Postdoc Conference*, Hradec Králové, Czechia
- **Šadibolová, M.**, Boušová, I., Juvonen, R., Auriola, S. (Sept 2020). Metabolism of helenalin in vitro and its interaction with human cytochrome P450 2A13. *25th Interdisciplinary Toxicology Conference*, Prague, Czechia
- **Šadibolová, M.**, Boušová, I., Juvonen, R., Auriola, S. (Jan 2021). Metabolism of helenalin in vitro and its interaction with human cytochrome P450. *11th Postgraduate and 9th Postdoc Conference*, Hradec Králové, Czechia

Posters:

- **Šadibolová, M.**, Zárybnický, T., Deingruberová, K., Ambrož, M., Šubrt, Z., Skálová, L., Boušová, I. (June 2018). Effect of cis-nerolidol, trans-nerolidol and farnesol on the mRNA and protein expression of phase I xenobiotic-metabolizing enzymes in precision-cut liver slices. *23rd Interdisciplinary Toxicology Conference*, Stará Lesná, Slovakia

8.2 Publications unrelated to the topic of the dissertation

- Vokřál, I., **Šadibolová, M.,** Podlipná, R., Lamka, J., Prchal, L., Sobotová, D., Lokvencová K., Szotáková, B., & Skálová, L. (2019). Ivermectin environmental impact: Excretion profile in sheep and phytotoxic effect in Sinapis alba. *Ecotoxicology and Environmental Safety*, *169*, 944–949.
- Ambrož, M., Šmatová, M., **Šadibolová, M.**, Pospíšilová, E., Hadravská, P., Kašparová, M., Skarková, V. H., Králová, V., & Skálová, L. (2019). Sesquiterpenes α-humulene and β-caryophyllene oxide enhance the efficacy of 5-fluorouracil and oxaliplatin in colon cancer cells. *Acta Pharmaceutica*, **69**(1), 121–128.

- Pavičić, A., Zajíčková, M., **Šadibolová, M.**, Svobodová, G., Matoušková, P., Szotáková, B., Langhansová, L., Maršík, P., & Skálová, L. (2023). Anthelmintic activity of European fern extracts against Haemonchus contortus. *Veterinary Research*, *54*(1), 59.

8.3 Presentations unrelated to the topic of the dissertation

Oral presentations:

- **Šadibolová, M.**, Vávrová, G., Omwanghe, E. A., Ambrož, M., Boušová, I. (Feb 2022). Changes in the expression of phase I-drug metabolizing enzymes in two mouse models of NAFLD. *12th Postgraduate and Postdoc Conference*, Hradec Králové, Czechia
- **Šadibolová, M.**, Horní, M., Huličiak, M., Prchal, L., Svobodová, G., Ambrož, M., Omwanghe, E. A., Boušová, I. (Feb 2023). Metabolism of 6-prenylnaringenin *in vitro*. *13th Postgraduate and Postdoc Conference*, Hradec Králové, Czechia

Posters:

- **Šadibolová, M.**, Svobodová, G., Omwanghe, E. A., Lenčo, J., Boušová, I. (Aug 2022). The hepatotoxicity of helenalin in differentiated HepaRG cells. *27th Interdisciplinary Toxicology Conference*, Hradec Králové, Czechia

8.4 Grant projects

Principal investigator:

- 2020-2022 Charles University Grant Agency (GA UK 1302120): The investigation of biotransformation of reactive sesquiterpenes and their effect on the proteome of human hepatic cells
- 2021-2022 START/MED/065 Novel therapeutic approaches to the treatment of hepatic diseases

Team member:

- 2018 Czech Science Foundation (Grant No. 18-07724S) Circulation of anthelmintics in environment - does it contribute to drug resistance development in helminths?
- 2018-2021 Czech Science Foundation (Grant No. 18-09946S) Metabolism of terpenes and mechanisms of their toxic effects in human liver

- 2023 Czech Science Foundation (Grant No. 20-14581Y) Transcriptomic, proteomic and functional analysis of carbonyl reductases in parasitic nematode *Haemonchus contortus*

8.5 International scientific experience

- Nov 2022 – June 2023: 8-month internship at the Core Facility for Mass Spectrometry & Proteomics, Center for Molecular Biology at the Heidelberg University, Heidelberg, Germany, under the supervision of Thomas Ruppert, Ph.D. and Marcin Luzarowski, Ph.D.
  - funded by project "Grant Schemes at CU" (reg. no. CZ.02.2.69/0.0/0.0/19_073/0016935) and Erasmus+
- Sept 2019 – Feb 2020: 6-month internship at the School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland, under the supervision of prof. Seppo Auriola and prof. Risto Juvonen
  - funded by Mobility Fund of Charles University (FM/c/2019-1-075)

8.6 Additional education

- Oct 2018: Advances in Molecular Biology and Genetics, Institute of Molecular Genetics, Czech Academy of Science, Prague, Czechia (5-day course)
- Feb 2019: 10th Hands-on Course in Ultrafast Sample Treatment for Proteomics, Universidade NOVA de Lisboa, Lisbon, Portugal (3-day course)
- Sept 2020: 21st School of Mass Spectrometry, Ioannes Marcus Marci Spectroscopic Society (IMMSS), Srní, Czechia (5-day course)
- June 2021: 12th International Summer School on Computational Mass Spectrometry-Based Proteomics, Max Planck Institute of Biochemistry (5-day summer school, online)
- Sept 2022: 13th International Summer School on Computational Mass Spectrometry-Based Proteomics, Barcelona, Spain (5-day summer school)

8.7 Teaching experience

Consultant of undergraduate students:

- Deingruberová, K. (2019). Modulatory effect of cis-nerolidol, trans-nerolidol and farnesol on selected phase 1 drug-metabolizing enzymes in human liver slices. Master's thesis
- Švábová, T. (2020). Effect of monoterpene citral on the expression of detoxification enzymes in HepaRG cells. Master's thesis
- Smolíková, M. (2021). Effect of camphor isomers on the expression of drug-metabolizing enzymes in human liver cells. Master's thesis
- Velecká, E. (2023). Effect of xanthohumol and 6-prenylnaringenin in the in vitro model of non-alcoholic fatty liver disease. Master's thesis
- Horní, M. (2023). Metabolism and transport of 6-prenylnaringenin *in vitro*. Master's thesis

Lectures and classes:

- 2017-2023 Czech and English practical courses of General Biochemistry
- 2022 Czech lecture on the topic: Xenobiotics – overview, toxicity, fate in organism; subject Xenobiochemistry

9 LIST OF ABBREVIATIONS

ABC	ATP-binding cassette
AhR	aryl hydrocarbon receptor
AhRR	aryl hydrocarbon receptor repressor
AKR	aldo-keto reductase
AMPK	5′-adenosine monophosphate-activated protein kinase
ARE	antioxidant response elements
ARNT	aryl hydrocarbon receptor nuclear translocator
bHLH-PAS	basic helix-loop-helix Per-Arnt-Single minded
BROD	benzoxyresorufin-*O*-dealkylase
CAR	constitutive androstane receptor
CYP	cytochrome P450
DMEs	drug-metabolizing enzymes
EROD	ethoxyresorufin-*O*-deethylase
GST	glutathione-*S*-transferase
Hsp90	heat shock protein 90
IC_{50}	half-maximal inhibitory concentration
JNK	c-Jun N-terminal kinase
Keap1	Kelch-like ECH-associated protein 1
MRP	multidrug resistance-associated protein
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NQO	NAD(P)H:quinone oxidoreductase
Nrf2	nuclear factor erythroid 2-related factor 2
PAH	polycyclic aromatic hydrocarbons
PP2A	protein phosphatase 2A
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator-1 alpha
PXR	pregnane X receptor
RXR	retinoid X receptor
SRC	steroid receptor coactivator
STAT3	signal transducer and activator of transcription 3
SULT	sulfotransferase
UGT	UDP-glucuronosyltransferase
XRE	xenobiotic responsive element

10 REFERENCES

The list of 189 references mentioned in the dissertation.

Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, C. R., Davis, R. J., & Ronai, Z. (1999). Regulation of JNK signaling by GSTp. *EMBO J*, *18*(5), 1321-1334. https://doi.org/10.1093/emboj/18.5.1321

Agbabiaka, T. B., Wider, B., Watson, L. K., & Goodman, C. (2017). Concurrent Use of Prescription Drugs and Herbal Medicinal Products in Older Adults: A Systematic Review. *Drugs Aging*, *34*(12), 891-905. https://doi.org/10.1007/s40266-017-0501-7

Albini, A., Dell'Eva, R., Vene, R., Ferrari, N., Buhler, D. R., Noonan, D. M., & Fassina, G. (2006). Mechanisms of the antiangiogenic activity by the hop flavonoid xanthohumol: NF-kappaB and Akt as targets. *FASEB J*, *20*(3), 527-529. https://doi.org/10.1096/fj.05-5128fje

Allocati, N., Masulli, M., Di Ilio, C., & Federici, L. (2018). Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. *Oncogenesis*, *7*(1), 8. https://doi.org/10.1038/s41389-017-0025-3

Almazroo, O. A., Miah, M. K., & Venkataramanan, R. (2017). Drug Metabolism in the Liver. *Clin Liver Dis*, *21*(1), 1-20. https://doi.org/10.1016/j.cld.2016.08.001

Amai, K., Fukami, T., Ichida, H., Watanabe, A., Nakano, M., Watanabe, K., & Nakajima, M. (2020). Quantitative analysis of mRNA expression levels of aldo-keto reductase and short-chain dehydrogenase/reductase isoforms in human livers. *Drug Metab Pharmacokinet*, *35*(6), 539-547. https://doi.org/10.1016/j.dmpk.2020.08.004

Aminkeng, F., Ross, C. J., Rassekh, S. R., Hwang, S., Rieder, M. J., Bhavsar, A. P., Smith, A., Sanatani, S., Gelmon, K. A., Bernstein, D., Hayden, M. R., Amstutz, U., Carleton, B. C., & Group, C. C. P. R. (2016). Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity. *Br J Clin Pharmacol*, *82*(3), 683-695. https://doi.org/10.1111/bcp.13008

Anderson, M. E. (1998). Glutathione: an overview of biosynthesis and modulation. *Chem Biol Interact*, *111-112*, 1-14. https://doi.org/10.1016/s0009-2797(97)00146-4

Anzenbacher, P., & Anzenbacherova, E. (2001). Cytochromes P450 and metabolism of xenobiotics. *Cell Mol Life Sci*, *58*(5-6), 737-747. https://doi.org/10.1007/pl00000897

Anzenbacher, P., & Zanger, U. M. (2012). *Metabolism of drugs and other xenobiotics*. Wiley. https://doi.org/10.1002/9783527630905

Badal, S., & Delgoda, R. (2014). Role of the modulation of CYP1A1 expression and activity in chemoprevention. *J Appl Toxicol*, *34*(7), 743-753. https://doi.org/10.1002/jat.2968

Baer-Dubowska, W., & Szaefer, H. (2013). Modulation of carcinogen-metabolizing cytochromes P450 by phytochemicals in humans. *Expert Opin Drug Metab Toxicol*, *9*(8), 927-941. https://doi.org/10.1517/17425255.2013.795219

Bai, X., Chen, Y., Hou, X., Huang, M., & Jin, J. (2016). Emerging role of NRF2 in chemoresistance by regulating drug-metabolizing enzymes and efflux transporters. *Drug Metab Rev*, *48*(4), 541-567. https://doi.org/10.1080/03602532.2016.1197239

Bailey, D. G., Dresser, G. K., Leake, B. F., & Kim, R. B. (2007). Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. *Clin Pharmacol Ther*, *81*(4), 495-502. https://doi.org/10.1038/sj.clpt.6100104

Barski, O. A., Tipparaju, S. M., & Bhatnagar, A. (2008). The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. *Drug Metab Rev*, *40*(4), 553-624. https://doi.org/10.1080/03602530802431439

Bartikova, H., Hanusova, V., Skalova, L., Ambroz, M., & Bousova, I. (2014). Antioxidant, pro-oxidant and other biological activities of sesquiterpenes. *Curr Top Med Chem*, *14*(22), 2478-2494. https://doi.org/10.2174/1568026614666141203120833

Board, P. G., & Menon, D. (2013). Glutathione transferases, regulators of cellular metabolism and physiology. *Biochim Biophys Acta*, *1830*(5), 3267-3288. https://doi.org/10.1016/j.bbagen.2012.11.019

Bock, K. W., & Kohle, C. (2005). UDP-glucuronosyltransferase 1A6: structural, functional, and regulatory aspects. *Methods Enzymol*, *400*, 57-75. https://doi.org/10.1016/S0076-6879(05)00004-2

Bolton, J. L., Dunlap, T. L., Hajirahimkhan, A., Mbachu, O., Chen, S. N., Chadwick, L., Nikolic, D., van Breemen, R. B., Pauli, G. F., & Dietz, B. M. (2019). The Multiple Biological Targets of Hops and Bioactive Compounds. *Chem Res Toxicol*, *32*(2), 222-233. https://doi.org/10.1021/acs.chemrestox.8b00345

Boonruang, S., Prakobsri, K., Pouyfung, P., Srisook, E., Prasopthum, A., Rongnoparut, P., & Sarapusit, S. (2017). Inhibition of human cytochromes P450 2A6 and 2A13 by flavonoids, acetylenic thiophenes and sesquiterpene lactones from Pluchea indica and Vernonia cinerea. *J Enzyme Inhib Med Chem*, *32*(1), 1136-1142. https://doi.org/10.1080/14756366.2017.1363741

Borrelli, F., & Izzo, A. A. (2009). Herb-drug interactions with St John's wort (Hypericum perforatum): an update on clinical observations. *AAPS J*, *11*(4), 710-727. https://doi.org/10.1208/s12248-009-9146-8

Bukum, N., Novotna, E., Morell, A., Hofman, J., & Wsol, V. (2019). Buparlisib is a novel inhibitor of daunorubicin reduction mediated by aldo-keto reductase 1C3. *Chem Biol Interact*, *302*, 101-107. https://doi.org/10.1016/j.cbi.2019.01.026

Bushey, R. T., Dluzen, D. F., & Lazarus, P. (2013). Importance of UDP-glucuronosyltransferases 2A2 and 2A3 in tobacco carcinogen metabolism. *Drug Metab Dispos*, *41*(1), 170-179. https://doi.org/10.1124/dmd.112.049171

Caputi, L., & Aprea, E. (2011). Use of terpenoids as natural flavouring compounds in food industry. *Recent Pat Food Nutr Agric*, *3*(1), 9-16. https://doi.org/10.2174/2212798411103010009

Cederbaum, A. I. (2015). Molecular mechanisms of the microsomal mixed function oxidases and biological and pathological implications. *Redox Biol*, *4*, 60-73. https://doi.org/10.1016/j.redox.2014.11.008

Cobbina, E., & Akhlaghi, F. (2017). Non-alcoholic fatty liver disease (NAFLD) - pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. *Drug Metab Rev*, *49*(2), 197-211. https://doi.org/10.1080/03602532.2017.1293683

Coleman, M. D. (2020). *Human Drug Metabolism*. Wiley. https://books.google.cz/books?id=8X7CDwAAQBAJ

Coughtrie, M. W. H. (2016). Function and organization of the human cytosolic sulfotransferase (SULT) family. *Chem Biol Interact*, *259*(Pt A), 2-7. https://doi.org/10.1016/j.cbi.2016.05.005

Croom, E. (2012). Metabolism of xenobiotics of human environments. *Prog Mol Biol Transl Sci*, *112*, 31-88. https://doi.org/10.1016/B978-0-12-415813-9.00003-9

D'Uva, G., Baci, D., Albini, A., & Noonan, D. M. (2018). Cancer chemoprevention revisited: Cytochrome P450 family 1B1 as a target in the tumor and the microenvironment. *Cancer Treat Rev*, *63*, 1-18. https://doi.org/10.1016/j.ctrv.2017.10.013

de Graaf, I. A., Groothuis, G. M., & Olinga, P. (2007). Precision-cut tissue slices as a tool to predict metabolism of novel drugs. *Expert Opin Drug Metab Toxicol*, *3*(6), 879-898. https://doi.org/10.1517/17425255.3.6.879

de Graaf, I. A., Olinga, P., de Jager, M. H., Merema, M. T., de Kanter, R., van de Kerkhof, E. G., & Groothuis, G. M. (2010). Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. *Nat Protoc*, *5*(9), 1540-1551. https://doi.org/10.1038/nprot.2010.111

de Lacerda Leite, G. M., de Oliveira Barbosa, M., Lopes, M. J. P., de Araújo Delmondes, G., Bezerra, D. S., Araújo, I. M., de Alencar, C. D. C., Coutinho, H. D. M., Peixoto, L. R., & Barbosa-Filho, J. M. (2021). Pharmacological and toxicological activities of α-humulene and its isomers: A systematic review. *Trends in Food Science & Technology*, *115*, 255-274.

Dietz, B. M., Hagos, G. K., Eskra, J. N., Wijewickrama, G. T., Anderson, J. R., Nikolic, D., Guo, J., Wright, B., Chen, S. N., Pauli, G. F., van Breemen, R. B., & Bolton, J. L. (2013). Differential regulation of detoxification enzymes in hepatic and mammary tissue by hops (Humulus lupulus) in vitro and in vivo. *Mol Nutr Food Res*, *57*(6), 1055-1066. https://doi.org/10.1002/mnfr.201200534

Djordjevic, N., Ghotbi, R., Bertilsson, L., Jankovic, S., & Aklillu, E. (2008). Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. *Eur J Clin Pharmacol*, *64*(4), 381-385. https://doi.org/10.1007/s00228-007-0438-6

Doherty, M. M., & Charman, W. N. (2002). The mucosa of the small intestine: how clinically relevant as an organ of drug metabolism? *Clin Pharmacokinet*, *41*(4), 235-253. https://doi.org/10.2165/00003088-200241040-00001

Dokduang, H., Yongvanit, P., Namwat, N., Pairojkul, C., Sangkhamanon, S., Yageta, M. S., Murakami, Y., & Loilome, W. (2016). Xanthohumol inhibits STAT3 activation pathway leading to growth suppression and apoptosis induction in human cholangiocarcinoma cells. *Oncol Rep*, *35*(4), 2065-2072. https://doi.org/10.3892/or.2016.4584

Drogosz, J., & Janecka, A. (2019). Helenalin - A Sesquiterpene Lactone with Multidirectional Activity. *Curr Drug Targets*, *20*(4), 444-452. https://doi.org/10.2174/1389450119666181012125230

Drozdzik, M., Busch, D., Lapczuk, J., Muller, J., Ostrowski, M., Kurzawski, M., & Oswald, S. (2018). Protein Abundance of Clinically Relevant Drug-Metabolizing Enzymes in the Human Liver and Intestine: A Comparative Analysis in Paired Tissue Specimens. *Clin Pharmacol Ther*, *104*(3), 515-524. https://doi.org/10.1002/cpt.967

Eagles, S. K., Gross, A. S., & McLachlan, A. J. (2020). The Effects of Cruciferous Vegetable-Enriched Diets on Drug Metabolism: A Systematic Review and Meta-Analysis of Dietary Intervention Trials in Humans. *Clin Pharmacol Ther*, *108*(2), 212-227. https://doi.org/10.1002/cpt.1811

Ebert, B., Kisiela, M., & Maser, E. (2016). Transcriptional regulation of human and murine short-chain dehydrogenase/reductases (SDRs) - an in silico approach. *Drug Metab Rev*, *48*(2), 183-217. https://doi.org/10.3109/03602532.2016.1167902

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*, *4*, 177. https://doi.org/10.3389/fphar.2013.00177

Elferink, M. G., Olinga, P., van Leeuwen, E. M., Bauerschmidt, S., Polman, J., Schoonen, W. G., Heisterkamp, S. H., & Groothuis, G. M. (2011). Gene expression analysis of precision-cut human liver slices indicates stable expression of ADME-Tox related genes. *Toxicol Appl Pharmacol*, *253*(1), 57-69. https://doi.org/10.1016/j.taap.2011.03.010

Falero-Perez, J., Song, Y. S., Sorenson, C. M., & Sheibani, N. (2018). CYP1B1: A key regulator of redox homeostasis. *Trends Cell Mol Biol*, *13*, 27-45. https://www.ncbi.nlm.nih.gov/pubmed/30894785

Fasinu, P. S., Bouic, P. J., & Rosenkranz, B. (2012). An overview of the evidence and mechanisms of herb-drug interactions. *Front Pharmacol*, *3*, 69. https://doi.org/10.3389/fphar.2012.00069

Fayyaz, A., Makwinja, S., Auriola, S., Raunio, H., & Juvonen, R. O. (2018). Comparison of In Vitro Hepatic Scoparone 7-O-Demethylation between Humans and Experimental Animals. *Planta Med*, *84*(5), 320-328. https://doi.org/10.1055/s-0043-119886

Fidyt, K., Fiedorowicz, A., Strzadala, L., & Szumny, A. (2016). beta-caryophyllene and beta-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Med*, *5*(10), 3007-3017. https://doi.org/10.1002/cam4.816

Fritz, A., Busch, D., Lapczuk, J., Ostrowski, M., Drozdzik, M., & Oswald, S. (2019). Expression of clinically relevant drug-metabolizing enzymes along the human intestine and their correlation to drug transporters and nuclear receptors: An intra-subject analysis. *Basic Clin Pharmacol Toxicol*, *124*(3), 245-255. https://doi.org/10.1111/bcpt.13137

Fukami, T., Yokoi, T., & Nakajima, M. (2022). Non-P450 Drug-Metabolizing Enzymes: Contribution to Drug Disposition, Toxicity, and Development. *Annu Rev Pharmacol Toxicol*, *62*, 405-425. https://doi.org/10.1146/annurev-pharmtox-052220-105907

Guengerich, F. P. (2006). Cytochrome P450s and other enzymes in drug metabolism and toxicity. *AAPS J*, *8*(1), E101-111. https://doi.org/10.1208/aapsj080112

Hakkola, J., Hukkanen, J., Turpeinen, M., & Pelkonen, O. (2020). Inhibition and induction of CYP enzymes in humans: an update. *Arch Toxicol*, *94*(11), 3671-3722. https://doi.org/10.1007/s00204-020-02936-7

Hankinson, O. (2005). Role of coactivators in transcriptional activation by the aryl hydrocarbon receptor. *Arch Biochem Biophys*, *433*(2), 379-386. https://doi.org/10.1016/j.abb.2004.09.031

Harjumaki, R., Pridgeon, C. S., & Ingelman-Sundberg, M. (2021). CYP2E1 in Alcoholic and Non-Alcoholic Liver Injury. Roles of ROS, Reactive Intermediates and Lipid Overload. *Int J Mol Sci*, *22*(15). https://doi.org/10.3390/ijms22158221

Hashiesh, H. M., Sharma, C., Goyal, S. N., Sadek, B., Jha, N. K., Kaabi, J. A., & Ojha, S. (2021). A focused review on CB2 receptor-selective pharmacological properties and therapeutic potential of beta-caryophyllene, a dietary cannabinoid. *Biomed Pharmacother*, *140*, 111639. https://doi.org/10.1016/j.biopha.2021.111639

Hayes, J. D., Flanagan, J. U., & Jowsey, I. R. (2005). Glutathione transferases. *Annu Rev Pharmacol Toxicol*, *45*, 51-88. https://doi.org/10.1146/annurev.pharmtox.45.120403.095857

He, K., Iyer, K. R., Hayes, R. N., Sinz, M. W., Woolf, T. F., & Hollenberg, P. F. (1998). Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. *Chem Res Toxicol*, *11*(4), 252-259. https://doi.org/10.1021/tx970192k

Henderson, M. C., Miranda, C. L., Stevens, J. F., Deinzer, M. L., & Buhler, D. R. (2000). In vitro inhibition of human P450 enzymes by prenylated flavonoids from hops, Humulus lupulus. *Xenobiotica*, *30*(3), 235-251. https://doi.org/10.1080/004982500237631

Hoffmann, F., & Maser, E. (2007). Carbonyl reductases and pluripotent hydroxysteroid dehydrogenases of the short-chain dehydrogenase/reductase superfamily. *Drug Metab Rev*, *39*(1), 87-144. https://doi.org/10.1080/03602530600969440

Hofman, J., Malcekova, B., Skarka, A., Novotna, E., & Wsol, V. (2014). Anthracycline resistance mediated by reductive metabolism in cancer cells: the role of aldo-keto reductase 1C3. *Toxicol Appl Pharmacol*, *278*(3), 238-248. https://doi.org/10.1016/j.taap.2014.04.027

Horn, T. L., Long, L., Cwik, M. J., Morrissey, R. L., Kapetanovic, I. M., & McCormick, D. L. (2005). Modulation of hepatic and renal drug metabolizing enzyme activities in rats by subchronic administration of farnesol. *Chem Biol Interact*, *152*(2-3), 79-99. https://doi.org/10.1016/j.cbi.2005.02.006

Hou, M. Z., Chen, L. L., Chang, C., Zan, J. F., & Du, S. M. (2021). Pharmacokinetic and tissue distribution study of eight volatile constituents in rats orally administrated with the essential

oil of Artemisiae argyi Folium by GC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci*, *1181*, 122904. https://doi.org/10.1016/j.jchromb.2021.122904

Chai, X., Zeng, S., & Xie, W. (2013). Nuclear receptors PXR and CAR: implications for drug metabolism regulation, pharmacogenomics and beyond. *Expert Opin Drug Metab Toxicol*, *9*(3), 253-266. https://doi.org/10.1517/17425255.2013.754010

Chan, W. K., Tan, L. T., Chan, K. G., Lee, L. H., & Goh, B. H. (2016). Nerolidol: A Sesquiterpene Alcohol with Multi-Faceted Pharmacological and Biological Activities. *Molecules*, *21*(5). https://doi.org/10.3390/molecules21050529

Chapman, D. E., Holbrook, D. J., Chaney, S. G., Hall, I. H., & Lee, K. H. (1989). In vitro inhibition of mouse hepatic mixed-function oxidase enzymes by helenalin and alantolactone. *Biochem Pharmacol*, *38*(22), 3913-3923. https://doi.org/10.1016/0006-2952(89)90668-0

Chapman, D. E., Holbrook, D. J., Chaney, S. G., Hall, I. H., & Lee, K. H. (1991). In vivo and in vitro effects of helenalin on mouse hepatic microsomal cytochrome P450. *Biochem Pharmacol*, *41*(2), 229-235. https://doi.org/10.1016/0006-2952(91)90481-j

Chapman, D. E., Roberts, G. B., Reynolds, D. J., Grippo, A. A., Holbrook, D. J., Hall, I. H., Chaney, S. G., Chang, J., & Lee, K. H. (1988). Acute toxicity of helenalin in BDF1 mice. *Fundam Appl Toxicol*, *10*(2), 302-312. https://www.ncbi.nlm.nih.gov/pubmed/3356317

Chaves, J. S., Leal, P. C., Pianowisky, L., & Calixto, J. B. (2008). Pharmacokinetics and tissue distribution of the sesquiterpene alpha-humulene in mice. *Planta Med*, *74*(14), 1678-1683. https://doi.org/10.1055/s-0028-1088307

Chen, B. H., Wang, C. C., Hou, Y. H., Mao, Y. C., & Yang, Y. S. (2015). Mechanism of sulfotransferase pharmacogenetics in altered xenobiotic metabolism. *Expert Opin Drug Metab Toxicol*, *11*(7), 1053-1071. https://doi.org/10.1517/17425255.2015.1045486

Chen, Y., Tang, Y., Guo, C., Wang, J., Boral, D., & Nie, D. (2012). Nuclear receptors in the multidrug resistance through the regulation of drug-metabolizing enzymes and drug transporters. *Biochem Pharmacol*, *83*(8), 1112-1126. https://doi.org/10.1016/j.bcp.2012.01.030

Choi, D. W., Kim, S. Y., Kim, S. K., & Kim, Y. C. (2000). Factors involved in hepatic glutathione depletion induced by acute ethanol administration. *J Toxicol Environ Health A*, *60*(7), 459-469. https://doi.org/10.1080/00984100050079520

Christian, K., Lang, M., Maurel, P., & Raffalli-Mathieu, F. (2004). Interaction of heterogeneous nuclear ribonucleoprotein A1 with cytochrome P450 2A6 mRNA: implications for post-transcriptional regulation of the CYP2A6 gene. *Mol Pharmacol*, *65*(6), 1405-1414. https://doi.org/10.1124/mol.65.6.1405

Ingelman-Sundberg, M. (2001). Implications of polymorphic cytochrome P450-dependent drug metabolism for drug development. *Drug metabolism and disposition*, *29*(4), 570-573.

Ioannides, C. (2001). Xenobiotic Metabolism: An Overview. In *Enzyme Systems that Metabolise Drugs and Other Xenobiotics* (pp. 1-32). https://doi.org/https://doi.org/10.1002/0470846305.ch1

Isvoran, A., Peng, Y., Ceauranu, S., Schmidt, L., Nicot, A. B., & Miteva, M. A. (2022). Pharmacogenetics of human sulfotransferases and impact of amino acid exchange on Phase II drug metabolism. *Drug Discov Today*, *27*(11), 103349. https://doi.org/10.1016/j.drudis.2022.103349

Jancova, P., Anzenbacher, P., & Anzenbacherova, E. (2010). Phase II drug metabolizing enzymes. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, *154*(2), 103-116. https://doi.org/10.5507/bp.2010.017

Jefferies, H., Coster, J., Khalil, A., Bot, J., McCauley, R. D., & Hall, J. C. (2003). Glutathione. *ANZ J Surg*, *73*(7), 517-522. https://doi.org/10.1046/j.1445-1433.2003.02682.x

Jin, Y., & Penning, T. M. (2007). Aldo-keto reductases and bioactivation/detoxication. *Annu Rev Pharmacol Toxicol*, *47*, 263-292. https://doi.org/10.1146/annurev.pharmtox.47.120505.105337

Jodynis-Liebert, J., Murias, M., & Bloszyk, E. (2000). Effect of sesquiterpene lactones on antioxidant enzymes and some drug-metabolizing enzymes in rat liver and kidney. *Planta Med*, *66*(3), 199-205. https://doi.org/10.1055/s-2000-8566

Jung, Y. Y., Hwang, S. T., Sethi, G., Fan, L., Arfuso, F., & Ahn, K. S. (2018). Potential Anti-Inflammatory and Anti-Cancer Properties of Farnesol. *Molecules*, *23*(11). https://doi.org/10.3390/molecules23112827

Juvonen, R. O., Novak, F., Emmanouilidou, E., Auriola, S., Timonen, J., Heikkinen, A. T., Kublbeck, J., Finel, M., & Raunio, H. (2019). Metabolism of Scoparone in Experimental Animals and Humans. *Planta Med*, *85*(6), 453-464. https://doi.org/10.1055/a-0835-2301

Kassner, N., Huse, K., Martin, H. J., Godtel-Armbrust, U., Metzger, A., Meineke, I., Brockmoller, J., Klein, K., Zanger, U. M., Maser, E., & Wojnowski, L. (2008). Carbonyl reductase 1 is a predominant doxorubicin reductase in the human liver. *Drug Metab Dispos*, *36*(10), 2113-2120. https://doi.org/10.1124/dmd.108.022251

Kennedy, D. A., & Seely, D. (2010). Clinically based evidence of drug-herb interactions: a systematic review. *Expert Opin Drug Saf*, *9*(1), 79-124. https://doi.org/10.1517/14740330903405593

Kewley, R. J., Whitelaw, M. L., & Chapman-Smith, A. (2004). The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. *Int J Biochem Cell Biol*, *36*(2), 189-204. https://doi.org/10.1016/s1357-2725(03)00211-5

Kobayashi, K., Hashimoto, M., Honkakoski, P., & Negishi, M. (2015). Regulation of gene expression by CAR: an update. *Arch Toxicol*, *89*(7), 1045-1055. https://doi.org/10.1007/s00204-015-1522-9

Kontogiannidou, E., Demertzidou, V. P., Andreadis, D. A., Demiri, E., Ritzoulis, C., Zografos, A. L., & Fatouros, D. G. (2017). Evaluation of sesquiterpenes as permeation enhancers for a model macromolecule across human skin in vitro. *Journal of Drug Delivery Science and Technology*, *41*, 384-389.

Krajka-Kuzniak, V., Paluszczak, J., & Baer-Dubowska, W. (2013). Xanthohumol induces phase II enzymes via Nrf2 in human hepatocytes in vitro. *Toxicol In Vitro*, *27*(1), 149-156. https://doi.org/10.1016/j.tiv.2012.10.008

Larigot, L., Juricek, L., Dairou, J., & Coumoul, X. (2018). AhR signaling pathways and regulatory functions. *Biochim Open*, *7*, 1-9. https://doi.org/10.1016/j.biopen.2018.05.001

Lennard, L., & Wang, L. M. (2017). Methyltransferases. In *Biotransformation* (pp. 497-516). Elsevier Inc.

Li, Y., Zeng, Y., Huang, Q., Wen, S., Wei, Y., Chen, Y., Zhang, X., Bai, F., Lu, Z., Wei, J., & Lin, X. (2019). Helenalin from Centipeda minima ameliorates acute hepatic injury by protecting mitochondria function, activating Nrf2 pathway and inhibiting NF-kappaB activation. *Biomed Pharmacother*, *119*, 109435. https://doi.org/10.1016/j.biopha.2019.109435

Lin, X., Zhang, S., Huang, R., Wei, L., Tan, S., Liang, S., Tian, Y., Wu, X., Lu, Z., & Huang, Q. (2014). Helenalin attenuates alcohol-induced hepatic fibrosis by enhancing ethanol metabolism, inhibiting oxidative stress and suppressing HSC activation. *Fitoterapia*, *95*, 203-213. https://doi.org/10.1016/j.fitote.2014.03.020

Lison, D. (1999). Importance of biotransformation pathways for interpreting biological monitoring of exposure. *Toxicol Lett*, *108*(2-3), 91-97. https://doi.org/10.1016/s0378-4274(99)00077-6

Liu, D., Zhang, L., Duan, L., Wu, J., Hu, M., Liu, Z., & Wang, C. (2019). Potential of herb-drug/herb interactions between substrates and inhibitors of UGTs derived from herbal medicines. *Pharmacological research*, *150*, 104510.

Liu, H., Yang, G., Tang, Y., Cao, D., Qi, T., Qi, Y., & Fan, G. (2013). Physicochemical characterization and pharmacokinetics evaluation of beta-caryophyllene/beta-cyclodextrin inclusion complex. *Int J Pharm*, *450*(1-2), 304-310. https://doi.org/10.1016/j.ijpharm.2013.04.013

Liu, X., Sui, X., Zhang, C., Wei, K., Bao, Y., Xiong, J., Zhou, Z., Chen, Z., Wang, C., Zhu, H., & Tang, F. (2020). Glutathione S-transferase A1 suppresses tumor progression and indicates better prognosis of human primary hepatocellular carcinoma. *J Cancer*, *11*(1), 83-91. https://doi.org/10.7150/jca.36495

Lnenickova, K., Svobodova, H., Skalova, L., Ambroz, M., Novak, F., & Matouskova, P. (2018). The impact of sesquiterpenes beta-caryophyllene oxide and trans-nerolidol on xenobiotic-metabolizing enzymes in mice in vivo. *Xenobiotica*, *48*(11), 1089-1097. https://doi.org/10.1080/00498254.2017.1398359

Lu, Y., & Cederbaum, A. I. (2008). CYP2E1 and oxidative liver injury by alcohol. *Free Radic Biol Med*, *44*(5), 723-738. https://doi.org/10.1016/j.freeradbiomed.2007.11.004

Malatkova, P., Maser, E., & Wsol, V. (2010). Human carbonyl reductases. *Curr Drug Metab*, *11*(8), 639-658. https://doi.org/10.2174/138920010794233530

Malatkova, P., & Wsol, V. (2014). Carbonyl reduction pathways in drug metabolism. *Drug Metab Rev*, *46*(1), 96-123. https://doi.org/10.3109/03602532.2013.853078

Martignoni, M., Groothuis, G. M., & de Kanter, R. (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol*, *2*(6), 875-894. https://doi.org/10.1517/17425255.2.6.875

Meech, R., Hu, D. G., McKinnon, R. A., Mubarokah, S. N., Haines, A. Z., Nair, P. C., Rowland, A., & Mackenzie, P. I. (2019). The UDP-Glycosyltransferase (UGT) Superfamily: New Members, New Functions, and Novel Paradigms. *Physiol Rev*, *99*(2), 1153-1222. https://doi.org/10.1152/physrev.00058.2017

Mohana, K., & Achary, A. (2017). Human cytosolic glutathione-S-transferases: quantitative analysis of expression, comparative analysis of structures and inhibition strategies of isozymes involved in drug resistance. *Drug Metab Rev*, *49*(3), 318-337. https://doi.org/10.1080/03602532.2017.1343343

Moine, L., Rivoira, M., Diaz de Barboza, G., Perez, A., & Tolosa de Talamoni, N. (2018). Glutathione depleting drugs, antioxidants and intestinal calcium absorption. *World J Gastroenterol*, *24*(44), 4979-4988. https://doi.org/10.3748/wjg.v24.i44.4979

Mordente, A., Silvestrini, A., Martorana, G. E., Tavian, D., & Meucci, E. (2015). Inhibition of Anthracycline Alcohol Metabolite Formation in Human Heart Cytosol: A Potential Role for Several Promising Drugs. *Drug Metab Dispos*, *43*(11), 1691-1701. https://doi.org/10.1124/dmd.115.065110

Moujir, L., Callies, O., Sousa, P. M., Sharopov, F., & Seca, A. M. (2020). Applications of sesquiterpene lactones: a review of some potential success cases. *Applied Sciences*, *10*(9), 3001.

Mukai, R. (2018). Prenylation enhances the biological activity of dietary flavonoids by altering their bioavailability. *Biosci Biotechnol Biochem*, *82*(2), 207-215. https://doi.org/10.1080/09168451.2017.1415750

Mukai, R., Fujikura, Y., Murota, K., Uehara, M., Minekawa, S., Matsui, N., Kawamura, T., Nemoto, H., & Terao, J. (2013). Prenylation enhances quercetin uptake and reduces efflux in Caco-2 cells and enhances tissue accumulation in mice fed long-term. *J Nutr*, *143*(10), 1558-1564. https://doi.org/10.3945/jn.113.176818

Mulero-Navarro, S., & Fernandez-Salguero, P. M. (2016). New Trends in Aryl Hydrocarbon Receptor Biology. *Front Cell Dev Biol*, *4*, 45. https://doi.org/10.3389/fcell.2016.00045

Naccarato, M., Yoong, D., & Gough, K. (2012). A potential drug-herbal interaction between Ginkgo biloba and efavirenz. *J Int Assoc Physicians AIDS Care (Chic)*, *11*(2), 98-100. https://doi.org/10.1177/1545109711435364

Nakamura, Y., Yoshida, C., Murakami, A., Ohigashi, H., Osawa, T., & Uchida, K. (2004). Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes. *FEBS Lett*, *572*(1-3), 245-250. https://doi.org/10.1016/j.febslet.2004.07.042

Navarro, V. J., & Senior, J. R. (2006). Drug-related hepatotoxicity. *N Engl J Med*, *354*(7), 731-739. https://doi.org/10.1056/NEJMra052270

Nebert, D. W., & Russell, D. W. (2002). Clinical importance of the cytochromes P450. *Lancet*, *360*(9340), 1155-1162. https://doi.org/10.1016/S0140-6736(02)11203-7

Neve, E. P., & Ingelman-Sundberg, M. (2008). Intracellular transport and localization of microsomal cytochrome P450. *Anal Bioanal Chem*, *392*(6), 1075-1084. https://doi.org/10.1007/s00216-008-2200-z

Nguyen, L. T., Myslivecкova, Z., Szotakova, B., Spicakova, A., Lnenickova, K., Ambroz, M., Kubicek, V., Krasulova, K., Anzenbacher, P., & Skalova, L. (2017). The inhibitory effects of beta-caryophyllene, beta-caryophyllene oxide and alpha-humulene on the activities of the main drug-metabolizing enzymes in rat and human liver in vitro. *Chem Biol Interact*, *278*, 123-128. https://doi.org/10.1016/j.cbi.2017.10.021

Niwa, T., Narita, K., Okamoto, A., Murayama, N., & Yamazaki, H. (2019). Comparison of Steroid Hormone Hydroxylations by and Docking to Human Cytochromes P450 3A4 and 3A5. *J Pharm Pharm Sci*, *22*(1), 332-339. https://doi.org/10.18433/jpps30558

Niwa, T., Okamoto, A., Narita, K., Toyota, M., Kato, K., Kobayashi, K., & Sasaki, S. (2020). Comparison of steroid hormone hydroxylation mediated by cytochrome P450 3A subfamilies. *Arch Biochem Biophys*, *682*, 108283. https://doi.org/10.1016/j.abb.2020.108283

Oda, S., Fukami, T., Yokoi, T., & Nakajima, M. (2015). A comprehensive review of UDP-glucuronosyltransferase and esterases for drug development. *Drug Metab Pharmacokinet*, *30*(1), 30-51. https://doi.org/10.1016/j.dmpk.2014.12.001

Overk, C. R., Yao, P., Chadwick, L. R., Nikolic, D., Sun, Y., Cuendet, M. A., Deng, Y., Hedayat, A. S., Pauli, G. F., Farnsworth, N. R., van Breemen, R. B., & Bolton, J. L. (2005). Comparison of the in vitro estrogenic activities of compounds from hops (Humulus lupulus) and red clover (Trifolium pratense). *J Agric Food Chem*, *53*(16), 6246-6253. https://doi.org/10.1021/jf050448p

Pan, L., Becker, H., & Gerhauser, C. (2005). Xanthohumol induces apoptosis in cultured 40-16 human colon cancer cells by activation of the death receptor- and mitochondrial pathway. *Mol Nutr Food Res*, *49*(9), 837-843. https://doi.org/10.1002/mnfr.200500065

Pang, Y., Nikolic, D., Zhu, D., Chadwick, L. R., Pauli, G. F., Farnsworth, N. R., & van Breemen, R. B. (2007). Binding of the hop (Humulus lupulus L.) chalcone xanthohumol to cytosolic proteins in Caco-2 intestinal epithelial cells. *Mol Nutr Food Res*, *51*(7), 872-879. https://doi.org/10.1002/mnfr.200600252

Pant, A., Rondini, E. A., & Kocarek, T. A. (2019). Farnesol induces fatty acid oxidation and decreases triglyceride accumulation in steatotic HepaRG cells. *Toxicol Appl Pharmacol*, *365*, 61-70. https://doi.org/10.1016/j.taap.2019.01.003

Pavek, P. (2016). Pregnane X Receptor (PXR)-Mediated Gene Repression and Cross-Talk of PXR with Other Nuclear Receptors via Coactivator Interactions. *Front Pharmacol*, *7*, 456. https://doi.org/10.3389/fphar.2016.00456

Pelkonen, O., Turpeinen, M., Hakkola, J., Honkakoski, P., Hukkanen, J., & Raunio, H. (2008). Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch Toxicol*, *82*(10), 667-715. https://doi.org/10.1007/s00204-008-0332-8

Penning, T. M. (2015). The aldo-keto reductases (AKRs): Overview. *Chem Biol Interact*, *234*, 236-246. https://doi.org/10.1016/j.cbi.2014.09.024

Penning, T. M., Wangtrakuldee, P., & Auchus, R. J. (2019). Structural and Functional Biology of Aldo-Keto Reductase Steroid-Transforming Enzymes. *Endocr Rev*, *40*(2), 447-475. https://doi.org/10.1210/er.2018-00089

Petrossian, T. C., & Clarke, S. G. (2011). Uncovering the human methyltransferasome. *Mol Cell Proteomics*, *10*(1), M110 000976. https://doi.org/10.1074/mcp.M110.000976

Prakash, C., Zuniga, B., Song, C. S., Jiang, S., Cropper, J., Park, S., & Chatterjee, B. (2015). Nuclear Receptors in Drug Metabolism, Drug Response and Drug Interactions. *Nucl Receptor Res*, *2*. https://doi.org/10.11131/2015/101178

Pusztaszeri, M. P., Genta, R. M., & Cryer, B. L. (2007). Drug-induced injury in the gastrointestinal tract: clinical and pathologic considerations. *Nat Clin Pract Gastroenterol Hepatol*, *4*(8), 442-453. https://doi.org/10.1038/ncpgasthep0896

Radovic, B., Hussong, R., Gerhauser, C., Meinl, W., Frank, N., Becker, H., & Kohrle, J. (2010). Xanthohumol, a prenylated chalcone from hops, modulates hepatic expression of genes involved in thyroid hormone distribution and metabolism. *Mol Nutr Food Res*, *54 Suppl 2*, S225-235. https://doi.org/10.1002/mnfr.200900489

Ramamoorthy, A., Liu, Y., Philips, S., Desta, Z., Lin, H., Goswami, C., Gaedigk, A., Li, L., Flockhart, D. A., & Skaar, T. C. (2013). Regulation of microRNA expression by rifampin in human hepatocytes. *Drug Metab Dispos*, *41*(10), 1763-1768. https://doi.org/10.1124/dmd.113.052886

Raunio, H., & Rahnasto-Rilla, M. (2012). CYP2A6: genetics, structure, regulation, and function. *Drug Metabol Drug Interact*, *27*(2), 73-88. https://doi.org/10.1515/dmdi-2012-0001

Rautio, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Jarvinen, T., & Savolainen, J. (2008). Prodrugs: design and clinical applications. *Nat Rev Drug Discov*, *7*(3), 255-270. https://doi.org/10.1038/nrd2468

Ravindran, S., Basu, S., Surve, P., Lonsane, G., & Sloka, N. (2012). Significance of biotransformation in drug discovery and development. *J Biotechnol Biomater S*, *13*.

Rendic, S., & Guengerich, F. P. (2012). Contributions of human enzymes in carcinogen metabolism. *Chem Res Toxicol*, *25*(7), 1316-1383. https://doi.org/10.1021/tx300132k

Rigalli, J. P., Theile, D., Nilles, J., & Weiss, J. (2021). Regulation of PXR Function by Coactivator and Corepressor Proteins: Ligand Binding Is Just the Beginning. *Cells*, *10*(11). https://doi.org/10.3390/cells10113137

Riches, Z., Stanley, E. L., Bloomer, J. C., & Coughtrie, M. W. (2009). Quantitative evaluation of the expression and activity of five major sulfotransferases (SULTs) in human tissues: the SULT "pie". *Drug Metab Dispos*, *37*(11), 2255-2261. https://doi.org/10.1124/dmd.109.028399

Robledinos-Anton, N., Fernandez-Gines, R., Manda, G., & Cuadrado, A. (2019). Activators and Inhibitors of NRF2: A Review of Their Potential for Clinical Development. *Oxid Med Cell Longev*, *2019*, 9372182. https://doi.org/10.1155/2019/9372182

Romero, L., Andrews, K., Ng, L., O'Rourke, K., Maslen, A., & Kirby, G. (2006). Human GSTA1-1 reduces c-Jun N-terminal kinase signalling and apoptosis in Caco-2 cells. *Biochem J*, *400*(1), 135-141. https://doi.org/10.1042/BJ20060110

Rondini, E. A., Duniec-Dmuchowski, Z., & Kocarek, T. A. (2016). Nonsterol Isoprenoids Activate Human Constitutive Androstane Receptor in an Isoform-Selective Manner in

Primary Cultured Mouse Hepatocytes. *Drug Metab Dispos*, *44*(4), 595-604. https://doi.org/10.1124/dmd.115.068551

Rowland, A., Miners, J. O., & Mackenzie, P. I. (2013). The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int J Biochem Cell Biol*, *45*(6), 1121-1132. https://doi.org/10.1016/j.biocel.2013.02.019

Saito, A. Y., Sussmann, R. A., Kimura, E. A., Cassera, M. B., & Katzin, A. M. (2015). Quantification of nerolidol in mouse plasma using gas chromatography-mass spectrometry. *J Pharm Biomed Anal*, *111*, 100-103. https://doi.org/10.1016/j.jpba.2015.03.030

Seliger, J. M., Martin, H. J., Maser, E., & Hintzpeter, J. (2019). Potent inhibition of human carbonyl reductase 1 (CBR1) by the prenylated chalconoid xanthohumol and its related prenylflavonoids isoxanthohumol and 8-prenylnaringenin. *Chem Biol Interact*, *305*, 156-162. https://doi.org/10.1016/j.cbi.2019.02.031

Seliger, J. M., Misuri, L., Maser, E., & Hintzpeter, J. (2018). The hop-derived compounds xanthohumol, isoxanthohumol and 8-prenylnaringenin are tight-binding inhibitors of human aldo-keto reductases 1B1 and 1B10. *J Enzyme Inhib Med Chem*, *33*(1), 607-614. https://doi.org/10.1080/14756366.2018.1437728

Shanu-Wilson, J., Evans, L., Wrigley, S., Steele, J., Atherton, J., & Boer, J. (2020). Biotransformation: impact and application of metabolism in drug discovery. *ACS Medicinal Chemistry Letters*, *11*(11), 2087-2107.

Sharma, A., Patrick, B., Li, J., Sharma, R., Jeyabal, P. V., Reddy, P. M., Awasthi, S., & Awasthi, Y. C. (2006). Glutathione S-transferases as antioxidant enzymes: small cell lung cancer (H69) cells transfected with hGSTA1 resist doxorubicin-induced apoptosis. *Arch Biochem Biophys*, *452*(2), 165-173. https://doi.org/10.1016/j.abb.2006.04.006

Shimada, T. (2006). Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metab Pharmacokinet*, *21*(4), 257-276. https://doi.org/10.2133/dmpk.21.257

Singh, R. R., & Reindl, K. M. (2021). Glutathione S-Transferases in Cancer. *Antioxidants (Basel)*, *10*(5). https://doi.org/10.3390/antiox10050701

Smith, T., Majid, F., Eckl, V., & Reynolds, C. M. (2021). Herbal supplement sales in US increase by record-breaking 17.3% in 2020. *HerbalGram*, *131*, 52-65.

Spicakova, A., Szotakova, B., Dimunova, D., Mysliveckova, Z., Kubicek, V., Ambroz, M., Lnenickova, K., Krasulova, K., Anzenbacher, P., & Skalova, L. (2017). Nerolidol and Farnesol Inhibit Some Cytochrome P450 Activities but Did Not Affect Other Xenobiotic-Metabolizing Enzymes in Rat and Human Hepatic Subcellular Fractions. *Molecules*, *22*(4). https://doi.org/10.3390/molecules22040509

Staines, A. G., Sindelar, P., Coughtrie, M. W., & Burchell, B. (2004). Farnesol is glucuronidated in human liver, kidney and intestine in vitro, and is a novel substrate for UGT2B7 and UGT1A1. *Biochem J*, *384*(Pt 3), 637-645. https://doi.org/10.1042/BJ20040997

Stejskalova, L., Dvorak, Z., & Pavek, P. (2011). Endogenous and exogenous ligands of aryl hydrocarbon receptor: current state of art. *Curr Drug Metab*, *12*(2), 198-212. https://doi.org/10.2174/138920011795016818

Stern, S., Kurian, R., & Wang, H. (2022). Clinical Relevance of the Constitutive Androstane Receptor. *Drug Metab Dispos*, *50*(7), 1010-1018. https://doi.org/10.1124/dmd.121.000483

Stevens, J. F., & Page, J. E. (2004). Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry*, *65*(10), 1317-1330. https://doi.org/10.1016/j.phytochem.2004.04.025

Stierum, R., Gaspari, M., Dommels, Y., Ouatas, T., Pluk, H., Jespersen, S., Vogels, J., Verhoeckx, K., Groten, J., & van Ommen, B. (2003). Proteome analysis reveals novel proteins associated with proliferation and differentiation of the colorectal cancer cell line

Caco-2. *Biochim Biophys Acta*, *1650*(1-2), 73-91. https://doi.org/10.1016/s1570-9639(03)00204-8
Stulikova, K., Karabin, M., Nespor, J., & Dostalek, P. (2018). Therapeutic Perspectives of 8-Prenylnaringenin, a Potent Phytoestrogen from Hops. *Molecules*, *23*(3). https://doi.org/10.3390/molecules23030660
Su, T., Bao, Z., Zhang, Q. Y., Smith, T. J., Hong, J. Y., & Ding, X. (2000). Human cytochrome P450 CYP2A13: predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res*, *60*(18), 5074-5079. https://www.ncbi.nlm.nih.gov/pubmed/11016631
Suiko, M., Kurogi, K., Hashiguchi, T., Sakakibara, Y., & Liu, M. C. (2017). Updated perspectives on the cytosolic sulfotransferases (SULTs) and SULT-mediated sulfation. *Biosci Biotechnol Biochem*, *81*(1), 63-72. https://doi.org/10.1080/09168451.2016.1222266
Tan, K. P., Wang, B., Yang, M., Boutros, P. C., Macaulay, J., Xu, H., Chuang, A. I., Kosuge, K., Yamamoto, M., Takahashi, S., Wu, A. M., Ross, D. D., Harper, P. A., & Ito, S. (2010). Aryl hydrocarbon receptor is a transcriptional activator of the human breast cancer resistance protein (BCRP/ABCG2). *Mol Pharmacol*, *78*(2), 175-185. https://doi.org/10.1124/mol.110.065078
Tassaneeyakul, W., Guo, L. Q., Fukuda, K., Ohta, T., & Yamazoe, Y. (2000). Inhibition selectivity of grapefruit juice components on human cytochromes P450. *Arch Biochem Biophys*, *378*(2), 356-363. https://doi.org/10.1006/abbi.2000.1835
Tolson, A. H., & Wang, H. (2010). Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Adv Drug Deliv Rev*, *62*(13), 1238-1249. https://doi.org/10.1016/j.addr.2010.08.006
Tonelli, C., Chio, I. I. C., & Tuveson, D. A. (2018). Transcriptional Regulation by Nrf2. *Antioxid Redox Signal*, *29*(17), 1727-1745. https://doi.org/10.1089/ars.2017.7342
Turrini, E., Haenisch, S., Laechelt, S., Diewock, T., Bruhn, O., & Cascorbi, I. (2012). MicroRNA profiling in K-562 cells under imatinib treatment: influence of miR-212 and miR-328 on ABCG2 expression. *Pharmacogenet Genomics*, *22*(3), 198-205. https://doi.org/10.1097/FPC.0b013e328350012b
Venkatakrishnan, K., von Moltke, L. L., Obach, R. S., & Greenblatt, D. J. (2003). Drug metabolism and drug interactions: application and clinical value of in vitro models. *Curr Drug Metab*, *4*(5), 423-459. https://doi.org/10.2174/1389200033489361
Vogt, B. L., & Richie, J. P., Jr. (1993). Fasting-induced depletion of glutathione in the aging mouse. *Biochem Pharmacol*, *46*(2), 257-263. https://doi.org/10.1016/0006-2952(93)90412-p
Vrzal, R. (2021). Genetic and Enzymatic Characteristics of CYP2A13 in Relation to Lung Damage. *Int J Mol Sci*, *22*(22). https://doi.org/10.3390/ijms222212306
Walsh, A. A., Szklarz, G. D., & Scott, E. E. (2013). Human cytochrome P450 1A1 structure and utility in understanding drug and xenobiotic metabolism. *J Biol Chem*, *288*(18), 12932-12943. https://doi.org/10.1074/jbc.M113.452953
Walsh, J. S., & Miwa, G. T. (2011). Bioactivation of drugs: risk and drug design. *Annu Rev Pharmacol Toxicol*, *51*, 145-167. https://doi.org/10.1146/annurev-pharmtox-010510-100514
Wang, S., Dunlap, T. L., Howell, C. E., Mbachu, O. C., Rue, E. A., Phansalkar, R., Chen, S. N., Pauli, G. F., Dietz, B. M., & Bolton, J. L. (2016). Hop (Humulus lupulus L.) Extract and 6-Prenylnaringenin Induce P450 1A1 Catalyzed Estrogen 2-Hydroxylation. *Chem Res Toxicol*, *29*(7), 1142-1150. https://doi.org/10.1021/acs.chemrestox.6b00112

Wang, T., Arifoglu, P., Ronai, Z., & Tew, K. D. (2001). Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. *J Biol Chem*, *276*(24), 20999-21003. https://doi.org/10.1074/jbc.M101355200

Wang, Y. M., Ong, S. S., Chai, S. C., & Chen, T. (2012). Role of CAR and PXR in xenobiotic sensing and metabolism. *Expert Opin Drug Metab Toxicol*, *8*(7), 803-817. https://doi.org/10.1517/17425255.2012.685237

White, N. J. (2008). Qinghaosu (artemisinin): the price of success. *Science*, *320*(5874), 330-334. https://doi.org/10.1126/science.1155165

WHO. (2021). Key technical issues of herbal medicines with reference to interaction with other medicines.

Williams, A. C., & Barry, B. W. (2004). Penetration enhancers. *Adv Drug Deliv Rev*, *56*(5), 603-618. https://doi.org/10.1016/j.addr.2003.10.025

Williams, J. A., Ring, B. J., Cantrell, V. E., Jones, D. R., Eckstein, J., Ruterbories, K., Hamman, M. A., Hall, S. D., & Wrighton, S. A. (2002). Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos*, *30*(8), 883-891. https://doi.org/10.1124/dmd.30.8.883

Xiong, Y., Wen, S., Li, Y., Wei, Y., Fang, B., Li, C., Huang, Q., & Lin, X. (2022). Comprehensive analysis of transcriptomics and metabolomics to illustrate the underlying mechanism of helenalin against hepatic fibrosis. *Eur J Pharmacol*, *919*, 174770. https://doi.org/10.1016/j.ejphar.2022.174770

Yang, S. P., & Raner, G. M. (2005). Cytochrome P450 expression and activities in human tongue cells and their modulation by green tea extract. *Toxicol Appl Pharmacol*, *202*(2), 140-150. https://doi.org/10.1016/j.taap.2004.06.014

Yoshinari, K., Ueda, R., Kusano, K., Yoshimura, T., Nagata, K., & Yamazoe, Y. (2008). Omeprazole transactivates human CYP1A1 and CYP1A2 expression through the common regulatory region containing multiple xenobiotic-responsive elements. *Biochem Pharmacol*, *76*(1), 139-145. https://doi.org/10.1016/j.bcp.2008.04.005

Yu, A. M., Tian, Y., Tu, M. J., Ho, P. Y., & Jilek, J. L. (2016). MicroRNA Pharmacoepigenetics: Posttranscriptional Regulation Mechanisms behind Variable Drug Disposition and Strategy to Develop More Effective Therapy. *Drug Metab Dispos*, *44*(3), 308-319. https://doi.org/10.1124/dmd.115.067470

Yu, J., Wang, Y., & Ragueneau-Majlessi, I. (2022a). Pharmacokinetic Drug-Drug Interactions with Drugs Approved by the US Food and Drug Administration in 2020: Mechanistic Understanding and Clinical Recommendations. *Drug Metab Dispos*, *50*(1), 1-7. https://doi.org/10.1124/dmd.121.000401

Yu, J., Wang, Y., & Ragueneau-Majlessi, I. (2022b). Strong Pharmacokinetic Drug-Drug Interactions With Drugs Approved by the US Food and Drug Administration in 2021: Mechanisms and Clinical Implications. *Clin Ther*, *44*(11), 1536-1544. https://doi.org/10.1016/j.clinthera.2022.09.009

Yuan, Y., Qiu, X., Nikolic, D., Chen, S. N., Huang, K., Li, G., Pauli, G. F., & van Breemen, R. B. (2014). Inhibition of human cytochrome P450 enzymes by hops (Humulus lupulus) and hop prenylphenols. *Eur J Pharm Sci*, *53*, 55-61. https://doi.org/10.1016/j.ejps.2013.12.003

Zanger, U. M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther*, *138*(1), 103-141. https://doi.org/10.1016/j.pharmthera.2012.12.007

Zanoli, P., & Zavatti, M. (2008). Pharmacognostic and pharmacological profile of Humulus lupulus L. *J Ethnopharmacol*, *116*(3), 383-396. https://doi.org/10.1016/j.jep.2008.01.011

Zgorzynska, E., Dziedzic, B., & Walczewska, A. (2021). An Overview of the Nrf2/ARE Pathway and Its Role in Neurodegenerative Diseases. *Int J Mol Sci*, *22*(17). https://doi.org/10.3390/ijms22179592

Zhang, Z. Y., & Wong, Y. N. (2005). Enzyme kinetics for clinically relevant CYP inhibition. *Curr Drug Metab*, *6*(3), 241-257. https://doi.org/10.2174/1389200054021834

Zhou, S., Yung Chan, S., Cher Goh, B., Chan, E., Duan, W., Huang, M., & McLeod, H. L. (2005). Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. *Clin Pharmacokinet*, *44*(3), 279-304. https://doi.org/10.2165/00003088-200544030-00005

Zhou, S. F. (2008). Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. *Curr Drug Metab*, *9*(4), 310-322. https://doi.org/10.2174/138920008784220664

Zhu, B. T. (2002). 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*, *3*(3), 321-349. https://doi.org/10.2174/1389200023337586

Ziegler, R. G., Fuhrman, B. J., Moore, S. C., & Matthews, C. E. (2015). Epidemiologic studies of estrogen metabolism and breast cancer. *Steroids*, *99*(Pt A), 67-75. https://doi.org/10.1016/j.steroids.2015.02.015

Zolnierczyk, A. K., Maczka, W. K., Grabarczyk, M., Winska, K., Wozniak, E., & Aniol, M. (2015). Isoxanthohumol--Biologically active hop flavonoid. *Fitoterapia*, *103*, 71-82. https://doi.org/10.1016/j.fitote.2015.03.007

11 APPENDICES

Copies of the articles related to the topic of this dissertation.

I. **Šadibolová, M.**, Zárybnický, T., Smutný, T., Pávek, P., Šubrt, Z., Matoušková, P., Skálová, L., & Boušová, I. (2019). Sesquiterpenes Are Agonists of the Pregnane X Receptor but Do Not Induce the Expression of Phase I Drug-Metabolizing Enzymes in the Human Liver. *International Journal of Molecular Sciences*, *20*(18), 4562. **(IF_{2019} = 4.556, Q1)**

II. **Šadibolová, M.**, Juvonen, R. O., Auriola, S., & Boušová, I. (2022). In vitro metabolism of helenalin and its inhibitory effect on human cytochrome P450 activity. *Archives of Toxicology*, *96*(3), 793–808. **(IF_{2022} = 6.1, Q1 - first decile)**

III. Lněničková, K., **Šadibolová, M.**, Matoušková, P., Szotáková, B., Skálová, L., & Boušová, I. (2020). The modulation of phase II drug-metabolizing enzymes in proliferating and differentiated CaCo-2 cells by hop-derived prenylflavonoids. *Nutrients*, *12*(7), 2138. **(IF_{2020} = 5.719, Q1)**