

Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

## Pharmacology &amp; Therapeutics

journal homepage: [www.elsevier.com/locate/pharmthera](http://www.elsevier.com/locate/pharmthera)

## Dietary flavonoids in cancer therapy and prevention: Substrates and inhibitors of cytochrome P450 CYP1 enzymes

Vasilis P. Androutsopoulos<sup>a,c,\*</sup>, Athanasios Papakyriakou<sup>b</sup>, Dionisios Vourloumis<sup>b</sup>,  
Aristidis M. Tsatsakis<sup>a</sup>, Demetrios A. Spandidos<sup>c</sup>

<sup>a</sup> Laboratory of Forensic Sciences and Toxicology, University of Crete, Heraklion, Voutes, 71003, Greece

<sup>b</sup> Laboratory of Chemical Biology of Natural Products and Designed Molecules, Institute of Physical Chemistry, NCSR "Demokritos" 15310 Agia Paraskevi Attikis, Athens, Greece

<sup>c</sup> Laboratory of Clinical Virology, University of Crete, Heraklion, Voutes, 71409, Greece

## ARTICLE INFO

## Keywords:

Flavonoids  
Chemoprevention  
CYP1 enzymes  
Inhibition  
Metabolism

## ABSTRACT

Flavonoids are polyphenolic compounds that have attracted the attention of the scientific community as the hallmark molecules responsible for cancer prevention by a plethora of different mechanisms. One of their most important characteristics, responsible for their cancer preventive properties, is their interaction with cytochrome P450 CYP1 enzymes. Flavonoids have traditionally been described as CYP1 inhibitors due to the inhibition of carcinogenic product formation and consequent blockage of the initiation stage of carcinogenesis. However, mounting evidence indicate that flavonoids are also capable of acting as CYP1 substrates, undergoing bioactivation to more antiproliferative agents within cancer cells. In this review, a comprehensive summary of the two models is presented. Structural features responsible for CYP1 inhibition or substrate turnover are discussed and limitations as well as discrepancies between procarcinogen-activating and 7-ethoxyresorufin-inhibition assay systems are further explored *in vitro* and *in vivo*. Moreover, a thorough investigation of the substrate specificity of flavonoids for the active site of CYP1 enzymes is undertaken. Finally, issues concerning the bioavailability and metabolic fate of these compounds *in vivo* are addressed. Ultimately, the mode of flavonoid action, in terms of CYP1 inhibition or CYP1-mediated bioactivation, is dependent on the lipophilicity or hydrophilicity of each compound. The degree of hydroxylation or methoxylation of the A and B rings is the major factor which determines the accessibility to the tumor site, in terms of hepatic and intestinal metabolism, and the introduction of the molecules to the CYP1 active site, respectively.

© 2010 Elsevier Inc. All rights reserved.

## Contents

1. Introduction . . . . .	10
2. Flavonoids as CYP1 inhibitors . . . . .	10
3. Flavonoids as CYP1 substrates . . . . .	12
4. Ligand-binding interactions of flavonoids with CYP1 enzymes . . . . .	13
5. Bioavailability of dietary flavonoids-CYP1 activation in cancer therapy and prevention . . . . .	15
6. Future considerations—Potential of methoxylated flavones in chemoprevention . . . . .	17
7. Conclusion . . . . .	17
References . . . . .	18

**Abbreviations:** CYP1, cytochrome P450 1 family enzymes; B[a]P, benzo[a]pyrene; EROD, 7-ethoxyresorufin-O-deethylase; MROD, 7-methoxyresorufin-O-demethylase; SGLT, sodium dependent glucose transporter; LPH, lactate phlorizin hydrolase; SULT, sulfotransferase enzyme; UGT, Uridine-5'-diphospho-glucuronosyl transferase enzyme; diosmin, diosmetin 7-O-rhamnoside.

\* Corresponding author. Laboratory of Forensic Sciences and Toxicology, University of Crete, Heraklion, Voutes, 71003, Greece. Tel.: +30 2810 394870; fax: +30 2810 542098.

E-mail address: [vasilis\\_androutsopoulos@yahoo.com](mailto:vasilis_androutsopoulos@yahoo.com) (V.P. Androutsopoulos).

## 1. Introduction

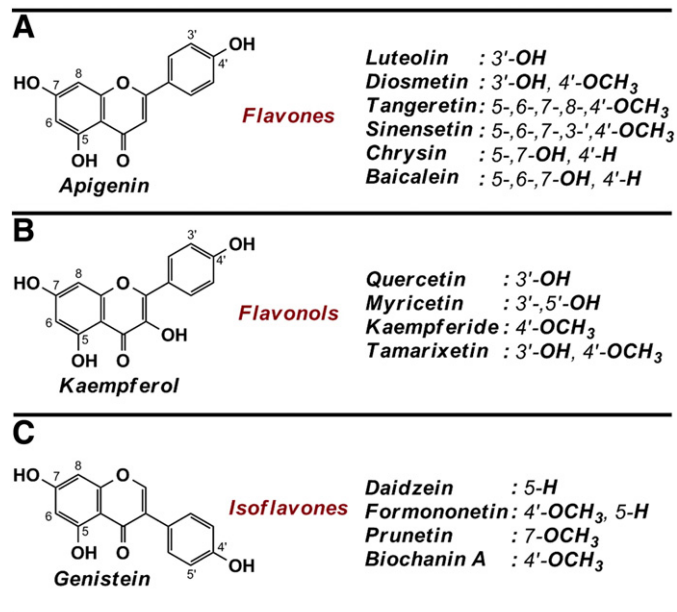
Flavonoids are naturally occurring polyphenolic compounds that constitute the most abundant class of dietary natural products and are present in fruits, vegetables, beverages and various dietary supplements, including herbal remedies such as Gingo Biloba and Milk Thistle, originating from medicinal plants. Extensive investigations of their bioactivity in the past 30 years have demonstrated their potential to prevent various diseases, such as cardiovascular disease, inflammatory disorders, viral infections, diabetes and neurological conditions (Scalbert & Williamson, 2000; Vaouzour et al., 2008; Rathee et al., 2009; Andres et al., 2009). Consequently, flavonoids are considered to be the key natural products that provide the most essential link between diet and the prevention of chronic disorders. Among the wide range of biochemical and pharmacological properties, one of their most investigated activities is their contribution to cancer prevention. Flavonoids can inhibit tumor formation and proliferation of cancer cells through various biological mechanisms of action. Particularly their effects on procarcinogen-activating enzymes, notably the cytochrome P450 CYP1 family, have been the focus of attention in cancer prevention during the last decade.

CYP1A1 and CYP1B1 enzymes have been shown to be over-expressed in tumors and metabolize procarcinogens to epoxide intermediates, which are further activated to diol epoxides by the enzyme epoxide hydrolase (Murray et al., 1995; Murray et al., 1997; Shimada & Fujii-Kuriyama, 2004). The most common chemical, extensively studied for its carcinogenicity, is Benzo[a]pyrene or B[a]P. Formation of B[a]P-7,8-diol-9,10-epoxides, referred to as bay region epoxides, causes accelerated DNA mutations due to the high reactivity of these chemicals. Any compound that interferes with this process, by blocking the formation of reactive intermediates, can potentially prevent the initiation of carcinogenesis. The ability of flavonoids to inhibit CYP1-enzymatic activity, and as a result CYP1-mediated formation of carcinogenic products, was established by various research groups (Ciolino et al., 1998; Ciolino & Yeh, 1999; Ciolino et al., 1999; Wen & Walle, 2005). In addition, it is becoming increasingly accepted that flavonoids may themselves be substrates for CYP1 enzymes and can cause inhibition of tumor cell growth by the formation of more pharmacologically active conversion products (Arroo et al., 2008, 2009; Androutsopoulos et al., 2009c).

The present review focuses on the flavonoid inhibitor and substrate interactions of the cytochrome P450 CYP1 family enzymes. Such interactions control entry of the latter compounds in the CYP1 active site and consequently regulate the mechanism by which the flavonoid–CYP1 chemopreventive effect is exerted. The structural features of the classes of flavonoids investigated in the present review are outlined in Fig. 1. Notably flavones such as apigenin, luteolin, diosmetin and tangeretin, flavonols such as myricetin, quercetin and kaempferol and isoflavones such as genistein and daidzein, are compounds abundant in dietary nutrients that are consumed daily worldwide. Thus, these compounds have been predominantly investigated, in terms of their abilities to interact with the cytochrome P450 CYP1 family of enzymes.

## 2. Flavonoids as CYP1 inhibitors

The potential of dietary flavonoids to inhibit CYP1 enzymatic activity has been demonstrated by numerous studies. The majority of the information in the literature comes from in vitro experimental data. Inhibition of *O*-deethylation of 7-ethoxyresorufin has been employed as a model assay for the determination of CYP1 inhibitory capacity of certain dietary flavonoids. Studies in the mid and late 1990s have shown the ability of these compounds to inhibit EROD and MROD activities in human and rat liver microsomes (Siess et al., 1995; Zhai et al., 1998a,b). These studies were focused on the inhibition of CYP1A2 by flavonoids, since only negligible expression of the extrahepatic



**Fig. 1.** Chemical structures of the three most important classes of flavonoids. **A.** Flavones. **B.** Flavonols. **C.** Isoflavones. Each flavonoid molecule is composed of three rings assigned **A, B** and **C** and varying number of hydroxyl or methoxy substitutions, which results in approximately eight thousand different compounds, based on the same core structure. Dietary products containing flavonoids are citrus fruits (tangeretin, sinensetin, and nobiletin), soya products (genistein and daidzein) and various other vegetables and plants (luteolin, apigenin in green peppers and parsley).

enzymes CYP1A1 and CYP1B1 is observed in liver microsomes. The structural features of the flavonoid molecule, which were initially found to be responsible for this inhibition, were the C2–C3 double bond and the number of hydroxyl substitutions in the A ring. Di- and tri-hydroxylated flavonoids, such as chrysin and galangin were highlighted as potent inhibitors of CYP1A2 (Zhai et al., 1998a). Subsequent studies in recombinant human CYP1A1 and CYP1A2 enzymes have corroborated the initial findings on flavonoid CYP1A inhibitory activity, since the flavone apigenin, which can be regarded as the 4'-hydroxylated analogue of chrysin and the flavone acacetin or 4'-methoxy chrysin, were shown to inhibit strongly CYP1A1 and CYP1A2 enzymatic activities (Pastrakuljic et al., 1997; Doostdar et al., 2000). Zhai et al. (1998a) further reported that galangin (3-hydroxy chrysin) was the most potent inhibitor of CYP1A2, whereas 7-hydroxyflavone exhibited the greatest selectivity for CYP1A1 in a small series of hydroxylated flavonoids. Flavones containing the C2–C3 double bond were more potent CYP1 inhibitors as opposed to flavonones lacking the specific functionality (Doostdar et al., 2000). Moreover, methoxy substitutions at the 4'-positions of the B ring of flavones or flavonones, in place of vacant or hydroxylated positions, enhanced further the inhibitory activity towards CYP1 enzymes (Doostdar et al., 2000).

Of note is that, compared to the CYP1A isozymes, CYP1B1 was more profoundly affected by acacetin and the structurally similar flavonoids diosmetin, naringenin, hesperitin, eriodictyol and homerioidictyol, as shown in a study undertaken by Doostdar et al. (2000). In agreement with these results, Gingo Biloba extract containing flavonoids, was demonstrated to inhibit CYP1B1-catalyzed EROD activity and Benzo[a]pyrene (B[a]P) hydroxylation to a greater extent, compared to CYP1A1 and CYP1A2 (Chang et al., 2006). The flavonols kaempferol, quercetin and isorhamnetin were highlighted as potent CYP1B1 inhibitors, representing the 3-hydroxylated equivalents of the flavones apigenin, luteolin and diosmetin, respectively. Luteolin was also reported to be a potent inhibitor of CYP1A1 by Kim et al. (2005), whereas chrysin exhibited the most pronounced effects of CYP1A2-dependent EROD inhibition. Thus, it appears that the strongest

inhibition of CYP1 enzyme-dependent-EROD activity is mainly due to flavones or flavonols with two or more hydroxyl groups.

Quercetin is one of the key flavonoids extensively demonstrated to inhibit CYP1A1 and CYP1B1 in enzyme- and cell-based assay models (Schwarz et al., 2003; Schwarz & Roots, 2003; Chaudhary & Willet, 2006; Leung et al., 2007). This molecule has attracted particular interest due to its abundance in nutrients which are common in the Western diet (Hollman & Katan, 1998). Quercetin was found to be a potent inhibitor of recombinant CYP1A1 and CYP1B1 EROD activity (Schwarz et al., 2003; Schwarz & Roots 2003; Chaudhary & Willet, 2006). 7-ethoxyresorufin-deethylase activity was also inhibited by quercetin at a concentration range of 0.5–5  $\mu\text{M}$  in 22Rv1 prostate cancer cells induced with TCDD, as well as in MCF-7<sub>CYP1A1</sub> cells, a stable CYP1A1 expressing clone of MCF-7 cells transfected with a pcDNA3.1 plasmid containing human CYP1A1 (Chaudhary & Willet, 2006; Leung et al., 2007). Compounds with a similar potency to that of quercetin in EROD and 7,8-diol-B[a]P epoxidation enzyme assays, include the flavonols myricetin, kaempferol and the flavone apigenin, all of which differ from quercetin in the presence or absence of an extra hydroxyl group at different positions (Schwarz et al., 2003; Chaudhary & Willet, 2006).

Although the EROD assay is a method widely accepted for flavonoid inhibitory interactions with CYP1 enzymes, it has been postulated that a strong EROD inhibition alone is insufficient to account for the classification of a particular compound, in terms of chemoprotection against CYP1 procarcinogen activation (Schwarz & Roots, 2003; Schwarz et al., 2005). The main reason for this hypothesis is the inconsistency in  $\text{IC}_{50}$  values of flavonoid inhibition on human CYP1A1 that has been noted by different assay models (Schwarz & Roots, 2003; Schwarz et al., 2005). For example kaempferol was 25 times less potent in inhibiting 7,8 diol-B[a]P epoxidation, as opposed to 7-ethoxyresorufin-O-deethylation, and inactive in inhibiting B[a]P metabolism in the Aryl hydrocarbon hydroxylase test (Schwarz & Roots, 2003).

Other studies have reported on the inhibiting effect of quercetin, chrysin and apigenin upon the induction of DNA damage caused by B[a]P and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in Chinese hamster ovary cells transfected with human and rat CYP1A1 and CYP1A2 (Lautraite et al., 2002). Quercetin was found to inhibit only rat CYP1A2 in this cell-based model, whereas chrysin and apigenin inhibited human CYP1A1 EROD activity as well as B[a]P-induced DNA damage (Lautraite et al., 2002). Although the study addressed the controversies between different assay systems in examining the inhibition of CYP1A enzymes by flavonoids, the overall findings were consistent with previous results in the literature as inhibition of CYP1A1 and CYP1A2 activities by chrysin, apigenin and quercetin correlated with the inhibition of DNA-adduct formation (Lautraite et al., 2002).

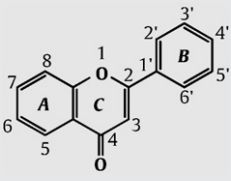
Studies undertaken by Walle and colleagues have found parallel results on the inhibitory action of 5,7-dimethoxy flavone by measuring B[a]P-DNA-binding and EROD activity (Wen & Walle, 2005; Wen et al., 2005). 5,7-dimethoxy flavone exhibited 50% inhibition of recombinant CYP1A1 and CYP1B1 EROD activity at submicromolar concentrations and 50 and 70% inhibition of B[a]P-DNA-binding at 2  $\mu\text{M}$  in HepG2 and SCC-9 cells respectively (Wen & Walle, 2005; Wen et al., 2005). Similarly, extensive profiling of the chemoprotective effect of the flavonol galangin by the group of Ciolino proved that it is an inhibitor of CYP1A1 since inhibition of EROD activity was demonstrated in isolated microsomes and intact MCF-7 cells induced by the carcinogen DMBA, along with inhibition of DMBA catabolism and DMBA-induced DNA-adduct formation in MCF-7 cells (Ciolino & Yeh, 1999). The  $\text{IC}_{50}$  noted when galangin was incubated with isolated microsomes ( $\approx 0.25 \mu\text{M}$ ) was smaller than the  $\text{IC}_{50}$  observed in the corresponding intact cells ( $\approx 1 \mu\text{M}$ ) and much smaller than the concentration required for 50% inhibition of DMBA-DNA-adduct formation ( $\approx 5 \mu\text{M}$ ). Nevertheless, the overall conclusion drawn was that galangin was an effective inhibitor of CYP1A1 in in vitro enzyme and cell-based assays.

Inhibition of CYP1 enzymatic activity has been reported for genistein, a prominent isoflavone with tumor suppressing activity, found primarily in nuts and soy-based products (Liggins et al., 2000; Mitropoulou et al., 2002; Nikitovic et al., 2003). Genistein demonstrated effective inhibition of CYP1 enzyme activity by EROD and the DMBA-induced DNA damage assay (Chan et al., 2003). Biochanin A, the 4' methoxylated derivative of genistein, was a more effective inhibitor of CYP1A1 and CYP1B1 enzymatic activities, as it was earlier noted for the flavone acacetin (Doostdar et al., 2000; Chan & Leung 2003; Chan et al., 2003). Substitution of a methoxy group at the 4'-position enhances the inhibition of the isoflavones towards the CYP1A1 and CYP1B1 enzymes. Further experiments in the MCF-7<sub>CYP1A1</sub> stable transfected cell line confirmed the potency of the isoflavones genistein and biochanin and the flavonols quercetin and kaempferol, demonstrated previously in recombinant CYP1 enzymes (Leung et al., 2007). In this cell-based assay model effective inhibition of EROD activity was reported for kaempferol and quercetin at submicromolar concentrations and for genistein and biochanin at 15 and 4  $\mu\text{M}$  respectively (Leung et al., 2007). Similar results were obtained for the isoflavone daidzein, with regard to CYP1A1 and CYP1B1 EROD inhibition, although to a lesser extent than either genistein or biochanin A (Chan and Leung 2003; Chan et al. 2003; Roberts et al., 2004; Leung et al., 2007). Overall the order of potency (biochanin A > genistein > daidzein) was consistent between different assay models (Roberts et al., 2004; Leung et al., 2007). The effects of hydroxyl and methoxy substitutions present in the flavonoid core structure on CYP1-EROD inhibition are outlined in Table 1.

In vivo data demonstrating direct CYP1 inhibitory activity of dietary flavonoids are very limited. The effect of grapefruit juice, which contains naringin, a glucoside of the flavonoid naringenin on the metabolism of caffeine by CYP1A2, was evaluated in a group of 12 healthy volunteers (Fuhr et al., 1993). A similar effect was noted for the isoflavone daidzein in a group of 20 volunteers with regard to CYP1A2 inhibition (Peng et al., 2003). The results showed that naringenin inhibits CYP1A2 in humans (Fuhr et al., 1993). The chemoprotective effect of several classes of flavonoids in vivo has been documented by studies in the early 1990s (Lamartiniere et al., 1995; Birt et al., 1997, 2001). Tumor development in mice or rats, that were treated with procarcinogens, such as DMBA, was inhibited following the administration of pure flavonoid compounds or dietary products (Lamartiniere et al., 1995; Birt et al., 1997, 2001). The exact mechanism of action remained unclear at that time. Some studies have suggested that cytochrome P450 inhibition could be one of the main mechanisms involved in the in vivo tumor reduction noted (Wei et al., 1998). This hypothesis is very likely to be true, since inhibition of EROD activity by quercetin was recently shown to occur ex vivo in lung microsomes of mice treated with benzo[a]pyrene. The authors suggested that the mechanism by which quercetin protects DNA damage caused by B[a]P could be related to CYP1A1 enzyme inhibition (Jin et al., 2006). Several studies have supported these findings with different classes of flavonoids tested each time, although a direct enzyme inhibition in mice or rats has yet to be demonstrated. Animals are usually treated with CYP1 inducers and the effects of flavonoids on the transcriptional repression of CYP1A1 and CYP1B1 genes in vivo are examined, or microsomes from various tissues are obtained following treatment, and the effects on CYP1 inhibition ex vivo are investigated (Helsby et al., 1998; Choi & Kim, 2008). To the best of our knowledge, the only study demonstrating this mode of action in vivo was performed in *S. cerevisiae* where inhibition of human CYP1A2 was possible by apigenin and quercetin. The authors reported lower  $\text{IC}_{50}$  values than those in microsomal preparations, due to the thickness of the cell wall affecting the permeability of each compound (Peterson et al., 2006). Although in vivo CYP1 EROD activity inhibition has not been demonstrated to date, the results presented in the literature suggest by indirect assays that in vivo inhibition of CYP1 enzymatic activity by dietary flavonoids is possible,



**Table 1**  
Effect of flavonoid substituents on the EROD inhibitory effect of CYP1 enzymes.



Substitution	Effect on CYP1 inhibition	Reference
7-methoxy (A ring)	–	Arroo et al., Dai et al.
6,7-dimethoxy (A ring)	–	Arroo et al.
Unsubstituted B ring	++	Zhai et al., Kim et al., Arroo et al., lori et al.
3'-hydroxy (B ring)	+/-	Arroo et al., Kim et al., Schwarz and Roots, lori et al.
4'-hydroxy (B ring)	–	lori et al., Schwarz and Roots, Arroo et al.
4'-methoxy (B ring)	++	Arroo et al., Doodstar et al., Chang et al., Leung et al., Roberts et al.
5'-hydroxy (B ring)	–	Arroo et al., Schwarz and Roots, Chaudhary and Willet
3-hydroxy (C-ring)	+	Leung et al., Arroo et al., Schwarz and Roots
C2–C3 double bond	++	Doodstar et al., Zhai et al., Schwarz and Roots

(+) Increased, (++) significantly increased, (–) decreased, (+/–) increased or decreased, depending on the CYP1 isoform.

thus accounting for the chemopreventive action of the latter compounds against procarcinogen activation.

### 3. Flavonoids as CYP1 substrates

Although the majority of the studies that investigated the interaction of flavonoids with CYP1 enzymes, in terms of their chemopreventive activity, points towards the procarcinogen activating inhibitory action, growing evidence suggest that flavonoids may also undergo CYP1-mediated oxidative metabolism to antiproliferative products. Early studies in the late 1990s examined the biotransformation of kaempferol to quercetin by rat CYP1A1. HPLC analysis of incubation of kaempferol with rat liver S9 mix showed the presence of a small metabolite, which was identified as quercetin (Silva et al., 1997). The authors highlighted that kaempferol can be a competitive inhibitor of CYP1A1 but, in doing so, it is transformed to the flavonol quercetin (Silva et al., 1997). Later studies by Breinholt confirmed that oxidative metabolism of dietary flavonoids by CYPs occurs in human liver microsomes, recombinant human enzymes and Aroclor 1254-induced rat liver microsomes (Breinholt et al., 2002, 2003). The involvement of CYP1A2 as a major contributor in the metabolism of the flavonones naringenin and hesperitin, the flavonols tamarixetin and kaempferol and the flavones apigenin and tangeretin was verified by inhibition studies, where potent inhibition by fluvoxamine, a selective CYP1A2 inhibitor, resulted in reduced formation of the corresponding hydroxylated metabolites (Breinholt et al., 2002, 2003). CYP3A4, CYP2C9 and CYP2D6 were also capable of catalyzing oxidative metabolism of some of the flavonoids tested, although to considerably smaller quantities than CYP1A2. The main metabolic routes of the CYP1A2-catalyzed oxidation involved 3'-hydroxylation and 4'-O-demethylation in the B ring. Interestingly, in the case of tangeretin, 6- and 7-O-demethylations in the A ring were observed. However, these reactions were only promoted by CYP3A4 (Breinholt et al., 2003). The conversion of apigenin to luteolin was also confirmed by Gradolatto et al. (2004) in a rat liver microsomal assay along with the presence of the two minor metabolites scutellarein (6-hydroxy apigenin) and isoscutellarein (8-hydroxy apigenin). The involvement of CYP2E1/3A and CYP2B/2C along with other hepatic

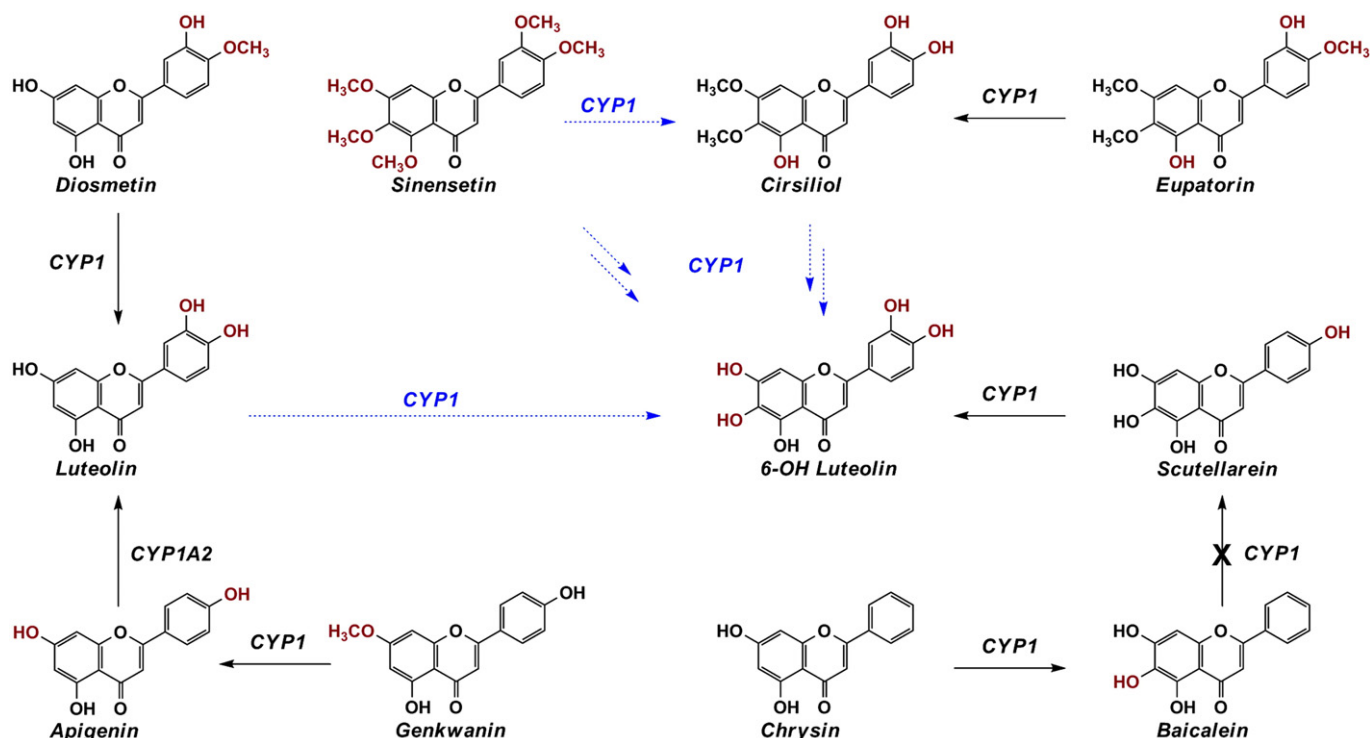
CYP isoforms was highlighted using specific inhibitors, although the effect of a selective CYP1A2 inhibitor was not investigated in that study (Gradolatto et al., 2004).

CYP1 bioconversions of dietary flavonoids were also established by previous studies undertaken in our laboratory. Rather than investigating the hepatic metabolism of such compounds, we have focused mainly on the aromatic hydroxylase and –O-demethylase activity of the extrahepatic enzymes CYP1A1 and CYP1B1. It was postulated that compounds which fall into the oestradiol pharmacophore, an endogenous CYP1 substrate, are hydroxylated by CYP1 enzymes in analogous unsubstituted positions (Potter et al., 2002). The first study which explored this possibility led to the conclusion that resveratrol, a stilbene found in red grapes, can be hydroxylated by CYP1B1 at the 3'-position to piceatannol (Potter et al., 2002). More recent discoveries have revealed that flavonoids containing methoxy groups are prone to oxidations catalyzed by CYP1 enzymes (Androutsopoulos et al., 2008; Androutsopoulos et al., 2009a,b,d,e). The flavone sinensetin, found in orange peel, which contains five methoxy groups, is rapidly metabolized by CYP1A1 and CYP1A2 (Androutsopoulos et al., 2009e). The flavones eupatorin, diosmetin and genkwanin are demethylated by CYP1 enzymes to the 4'-, 4'- and 7-positions respectively to yield the corresponding hydroxylated metabolites. The main metabolic route of catalysis for the methylated flavones diosmetin and eupatorin was demethylation in the B ring. Minor metabolites of the latter compounds were also present in vitro recombinant CYP1 incubates and in CYP1-expressing cell lines, leaving an option for possible hydroxylations in unsubstituted positions of the A ring. Hydroxylation in the unsubstituted 6-position of the A ring was noted for the flavone chrysin by the CYP1 family of enzymes, whereas scutellarein, the 6-hydroxylated equivalent of apigenin, was oxidized at the 3'-position to produce 6-hydroxy luteolin, as previously noted for the conversion of apigenin to luteolin by CYP1A2 (Breinholt et al., 2002; Androutsopoulos et al., 2009e).

With the exception of genkwanin demethylation to apigenin, CYP1B1 is the weakest catalyst, whereas CYP1A1 exhibits the highest affinity of flavonoid-oxidative metabolism, compared to the other CYP1 members (Androutsopoulos et al., 2008; Androutsopoulos et al., 2009a,b,d,e). These findings are in agreement with the studies undertaken by Walle and Walle (2007), whereby CYP1A1 was proven to be the fastest metabolizer of fully methylated flavones, notably tangeretin and sinensetin. The proposed biochemical pathway of CYP1-mediated-flavone/flavonol-bioconversions is shown in Figs. 2 and 3.

CYP1-catalyzed oxidative metabolism has also been noted for the isoflavones genistein and daidzein. Hydroxylations at the 3'-position of the B ring have been reported for both genistein and daidzein in human liver microsomes and recombinant CYP1 enzymes (Breinholt et al., 2003, Atherton et al., 2006). A third metabolite resulting from hydroxylation at the 6-position of the A ring of daidzein was observed after incubation of the compound with human liver microsomes and recombinant CYP1 enzymes, notably CYP1B1 and CYP1A2 (Atherton et al., 2006). In addition, genistein and daidzein are the conversion products of CYP1A2 oxidation of the methoxylated isoflavones biochanin A, formononetin, prunetin and 5-,4'-dimethoxyflavone (Hu et al., 2003). Fig. 4 shows the CYP1A2 and CYP1 metabolic pathways of the isoflavones studied thus far. Other CYPs capable of catalyzing 3'-hydroxylation of genistein to lesser extents are CYP2E1 and CYP2D6. It is noteworthy that 7-demethylation of prunetin by CYP1A2 is analogous to 7-demethylation of genkwanin by CYP1 enzymes.

In contrast to the previously proposed mechanism of action which involves CYP1-mediated inhibition of carcinogenic product formation, flavonoids are demonstrated in this way to undergo bioactivation by the latter enzymes to metabolites that inhibit tumor cell growth. In vivo results of dietary flavonoids exhibiting this mode of action have not been documented to date, although CYP1A1 was shown to activate a synthetic flavone, which has entered clinical phase I trial, in an MCF7 tumor xenograft by 4'-hydroxylation of the B ring (Loaiza-Pérez et al., 2004). In context of the toxicological significance of



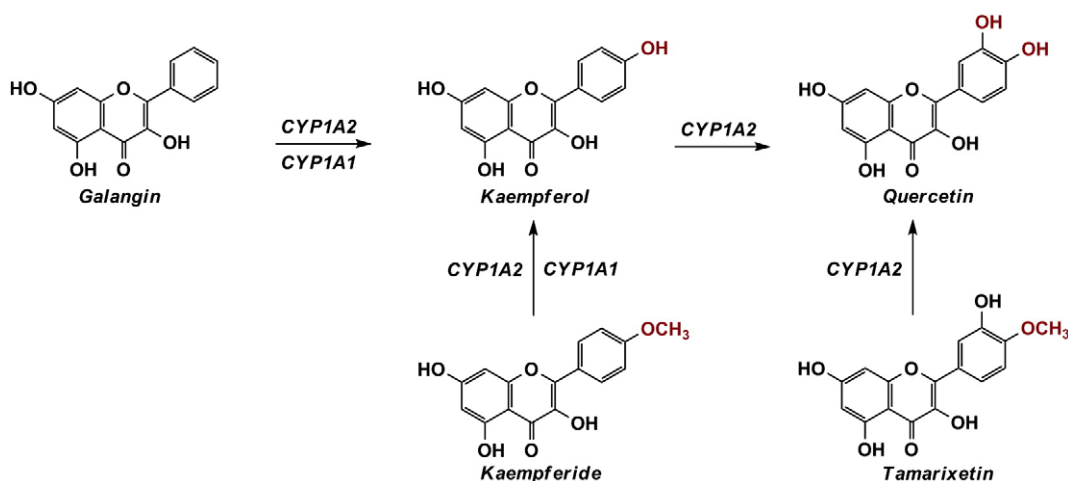
**Fig. 2.** Schematic diagram of the CYP1-mediated bioconversions of dietary flavones. It is interesting to note that fully methoxylated flavonoids, such as sinensetin may undergo subsequent one-step demethylations by CYP1 enzymes resulting in fully hydroxylated flavones, such as 6-OH luteolin. The same end product may result by hydroxylation reactions of poly-hydroxylated flavones, catalyzed by CYP1 enzymes. As methylation protects from extensive metabolism and masks potential bioactivity of flavonoids, the hydroxylated CYP1-conversion products are believed to possess increased antiproliferative and pharmacological activities. These results have been documented in *in vitro* enzyme and cell-based models (Androutsopoulos et al., 2008; Androutsopoulos et al., 2009a,b,d,e).

CYP1 enzyme-flavonoid activation *in vivo*, one study underlined that metabolism of the synthetic flavonoid ipriflavone or 6-O-isopropylflavone in rats is possible, to some extent, by CYP1A enzymes (Chung et al., 2006). The overall outcome of CYP1–flavonoid bioconversions is the enhancement of their antiproliferative or pharmacological activity in *in vitro* cell line and enzyme models.

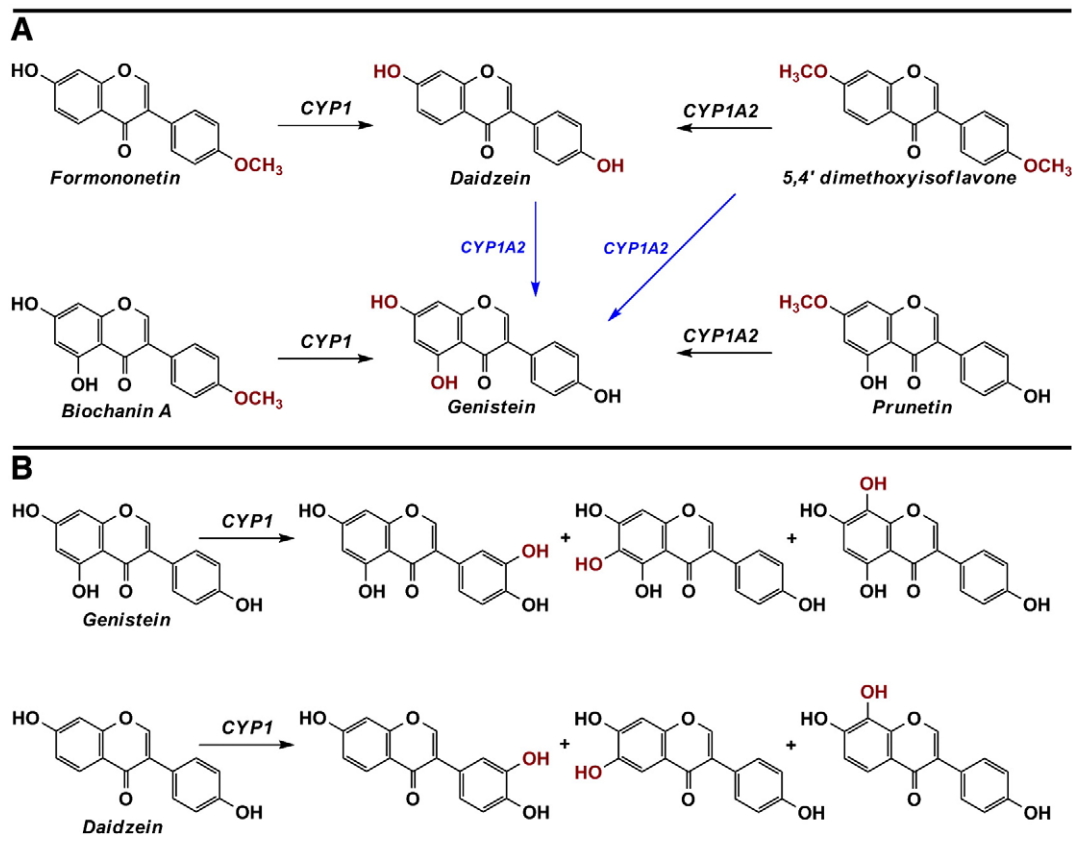
#### 4. Ligand-binding interactions of flavonoids with CYP1 enzymes

It is noteworthy that some dietary flavonoids, such as galangin and diosmetin, have been documented as CYP1 inhibitors by some studies

and as CYP1 substrates by others. In such cases, the possibility that a compound is acting with two different mechanisms must be considered, even though certain conventional assays produce non-homogeneous results, due to the difference in experimental test models and conditions used in each study. For example, Doostdar et al. (2000) demonstrated that diosmetin is a CYP1A1 and CYP1B1 selective inhibitor by inhibiting dealkylation of 7-ethoxyresorufin. However, a CYP1A1 and CYP1B1 selective substrate would also produce the same inhibitory profile. Ciolino et al. (1998) showed that diosmetin is a CYP1A1 non-competitive inhibitor in microsomes isolated from induced MCF-7 cells. The study did not examine whether metabolism



**Fig. 3.** CYP1-mediated metabolism of dietary flavonols. Starting with di-hydroxylated or partially methylated flavonols, such as galangin and tamarixetin, respectively, CYP1A1 and CYP1A2 enzymes are capable of catalyzing bioconversions to the tetra-hydroxylated end product quercetin, an abundant flavonol in the Western diet. Oxidations of galangin and kaempferide to kaempferol are also catalyzed by CYP2C9, whereas oxidation of tamarixetin to quercetin by CYP3A4 and CYP2D6 (Otake & Walle, 2002; Breinholt et al., 2002).



**Fig. 4. A.** CYP1-catalyzed conversions of methoxylated and hydroxylated isoflavones occur in analogous positions as previously reported for flavones and flavonols. Small captions (blue) for CYP1A2 indicate a weak catalysis for the hydroxylation of daidzein to genistein. **B.** Hydroxylation of the isoflavone genistein and daidzein is also possible at positions 6 and 8 of the A ring. Similar in vitro CYP1-routes of metabolism have been noted for the methylated isoflavone biochanin A.

of diosmetin by CYP1A1, or the other two members of the CYP1 family, would produce a metabolite which itself could act as an inhibitor. It was later shown that diosmetin is metabolized to a large extent by CYP1A1, CYP1A2 and to a lesser extent by CYP1B1 to the structurally related flavone luteolin (Androutsopoulos et al., 2009a). A similar finding was reported by Otake and Walle for the conversion of galangin to kaempferol by CYP1A1, which was initially established as a CYP1A2 and a CYP1A1 inhibitor (Otake & Walle 2002; Zhai et al., 1998a; Ciolino & Yeh, 1999). The distinction between the CYP1 inhibition concept and the CYP1 substrate activation concept is possible by flavonoid-metabolism studies. The conversion products arising from CYP1-oxidation of flavonoids account for substrate binding to the CYP1 active site.

Nevertheless, it is important to stress that the competitive inhibition of kaempferol and apigenin by CYP1A1 and CYP1A2 that were reported by Chang et al. and Zhai et al. respectively, as well as the mixed type inhibition of galangin by CYP1A1 and CYP1A2, reported by Zhai et al., account for putative substrate binding of these flavonoids to the heme group, although direct CYP1-oxidative metabolism was not investigated in these studies. Zhai et al. (1998a) have underlined that the mixed type of inhibition of galangin may be interpreted either by competition for binding to the active site (i.e. substrate binding), or by docking to a region that does not participate directly in substrate binding.

Consequently, a more detailed investigation of the active site of CYP1 enzymes is required to illuminate the possible discrepancies observed between the substrate and inhibitor propensities of the flavonoid structures. With this caveat in mind, molecular modeling and site-directed mutagenesis analyses have provided substantial information in terms of key amino acid residues involved in the catalytic binding of CYP1 enzymes with their corresponding substrates. Initial models for

CYP1A1 and CYP1A2, constructed on the basis of homology with the bacterial CYP102 by Lewis et al. (1999), have revealed that the amino acid residues critical for the substrate orientation to the heme pocket are essentially hydrophilic. In agreement with this study, Dai et al. (1998) highlighted in a previous report that although the active site of CYP1A2 is hydrophobic, significant hydrogen-bonding interactions are promoted between the residues Thr-385 and Arg-108 and the hydroxyl groups of flavone molecules. As a result, the flavonoid 3,5,7-tri-hydroxyflavone was predicted to bind more strongly to the active site at a perpendicular orientation due to increased hydrogen bonding, as opposed to flavone and 3,5,7-tri-methoxyflavone (Dai et al., 1998). This prediction was confirmed by in vitro inhibition experiments where 3,5,7-tri-hydroxyflavone was shown to have the highest affinity for the substrate binding pocket (Dai et al., 1998). Based upon these studies, site-directed mutagenesis analyses of the active site of human CYP1A2 suggested the importance of the residues Arg-108, Thr-385 and Thr-321 in the interaction of the enzyme with both substrates and inhibitors (Hadjokas et al., 2002). Substitution of Arg-108, Thr-385 and Thr-321 residues to Cys and Val residues respectively, produced recombinant CYP1A2 mutant proteins devoid of any enzyme activity (Hadjokas et al., 2002). The molecular model further predicted that the flavonol galangin would bind more strongly to the CYP1A2 active site, compared to flavone, due to additional hydrogen bonding of the 3-position of the C-ring with Thr-385, as noted earlier by Dai et al. (Hadjokas et al., 2002). Moreover, Liu et al. (2003) and Liu et al. (2004) identified Val-382 in the second CYP1A isoform, CYP1A1, as a key residue which affects the catalytic activity of the enzyme by molecular modeling based on the structure of CYP2C5 and site-directed mutagenesis studies. Mutants carrying substitutions of Val-382 with Ala or Leu had decreased EROD and MROD activities and 2-fold increased 7-pentoxylresorufin activity in the case of Ala mutations (Liu et al., 2003). Key factors responsible for the turnover of alkoxy-O-

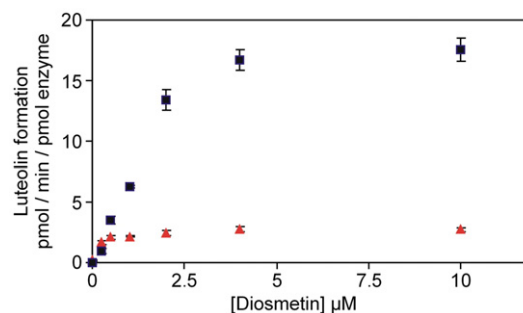
dealkylation of CYP1A1 and CYP1A2 included the substrate binding affinity, the substrate binding-induced reduction of the heme and the substrate binding orientation and mobility (Liu et al., 2004; Lewis et al., 2004, 2007). These two studies however, examined neither the catalytic specificity nor the binding of these mutants on flavone substrates.

Molecular modeling of several flavonoids, containing hydroxyl or methoxy groups, on CYP1A1 and CYP1A2 isozymes, based on homology of bacterial CYPs and the chimeric CYP2C5-2C3, has been performed by Iori and coworkers. The study further supported a strong network of hydrogen bonding which is required for binding to the active site (Iori et al., 2005). Flavonoids carrying several hydroxyl groups, such as quercetin and myricetin, were predicted to reside in close proximity to the heme site, leaving the catalytic site unperturbed (Iori et al., 2005). In contrast, kaempferol containing less hydroxyl groups than quercetin was shown to accumulate deeper into the CYP1A2 binding site with the pendant phenyl ring almost perpendicular to the heme (Iori et al., 2005). In this sense, it is tempting to assume that flavonoids containing multiple hydroxyl groups would be weaker substrates for CYP1A1/CYP1A2 enzymes, than those with less, due to their limited access to the heme pocket. This might also explain why scutellarein carrying four hydroxyl groups was not as efficient CYP1 substrate as chrysin, which contains two hydroxyl groups (Androutsopoulos et al., 2009e).

In addition to hydrogen-bonding,  $\pi$ - $\pi$  stacking interactions of CYP1 substrates with amino acid residues, such as Phe231, in close vicinity to the active site have been underlined as important contributors to the CYP1 substrate catalytic binding by Lewis et al. (2003) and Lewis et al. (1999). More importantly, CYP1B1 exhibits significant differences in the ligand orientation of several compounds which are CYP1 substrates and inhibitors, such as alkoxyresorufin, oestradiol and  $\alpha$ -naphthoflavone, compared to the other two isoforms, because several hydrogen-bond donor/acceptor side chains of the putative active site become essentially hydrophobic residues (Lewis et al., 1999; Don et al., 2003; Lewis et al., 2003).

Previous models of CYP1A1 or CYP1B1 by Lewis et al. or Iori et al., were based on the crystal structure of bacterial homologues and human P450s of the CYP2 family. The crystal structure of human CYP1A2 was published recently, enabling the construction of CYP1A1 and CYP1B1 homology models with greater sequence similarity than those reported in previous studies (Sansen et al., 2007). Such models can be used as tools for providing insight into the substrate orientation of dietary flavonoids to the extrahepatic CYP1B1 and CYP1A1 enzymes active site. Given that CYP1-mediated-flavonoid metabolic turnover can suppress tumor growth, whereas flavonoid-CYP1 inhibition can prevent initiation of carcinogenesis, molecular modeling analysis offers a mechanistic explanation that relates the structural features of dietary flavonoids with their cancer preventive or cancer therapeutic activities.

The 4-*O*-demethylation of diosmetin to luteolin was recently shown to be CYP1A1 selective by Michaelis–Menten kinetic analysis (Fig. 5, Androutsopoulos et al., 2009a). To provide plausible structure–activity relationships for the catalytic specificity of CYP1A1 and CYP1B1 towards dietary flavonoids we have employed molecular modeling based on the X-ray crystal structures of human CYP1A2 and CYP2C9 (Williams et al., 2003; Sansen et al., 2007). CYP1A1 model was built using CYP1A2 as the template, while CYP1B1 was based on both CYP1A2 and CYP2C9 X-ray structures (unpublished data). Docking of diosmetin within the active site of CYP1A1 and CYP1B1 (Fig. 6A and B, respectively) revealed a similar substrate orientation that brings the 4'-methoxy group in proximity to the heme iron, at  $\sim 4.5$  Å. The two lipophilic active sites accommodate the substrate providing contacts with  $>10$  non-polar residues and at least 4 hydrogen-bonding interactions. However, inspection of the generated models revealed two major differences in the diosmetin–CYP1 interactions: (a) the hydrogen-bonding interaction of O1 with Ser124 of CYP1A1 (numbering according to the CYP1A2 X-ray structure), which is replaced



**Fig. 5.** Michaelis–Menten enzyme kinetic analysis of diosmetin 4'-*O*-demethylation to luteolin by CYP1A1 and CYP1B1 (Androutsopoulos et al., 2009a). Triangles (red): CYP1B1, squares (blue): CYP1A1. Recombinant CYP1A1 and CYP1B1 microsomes were incubated at 37 °C for 10 min with diosmetin and the amount of luteolin formed measured by HPLC as described previously (Androutsopoulos et al., 2009a).

by Ala124 in CYP1B1 and (b) the stronger hydrophobic interaction between ring A and Leu314/Leu316 of CYP1A1 with respect to Thr314/Thr316 of CYP1B1. The higher affinity of CYP1A1 for diosmetin is reflected at the difference in the free energy of binding, which was predicted to be  $\sim 1.2$  kcal/mol (unpublished data).

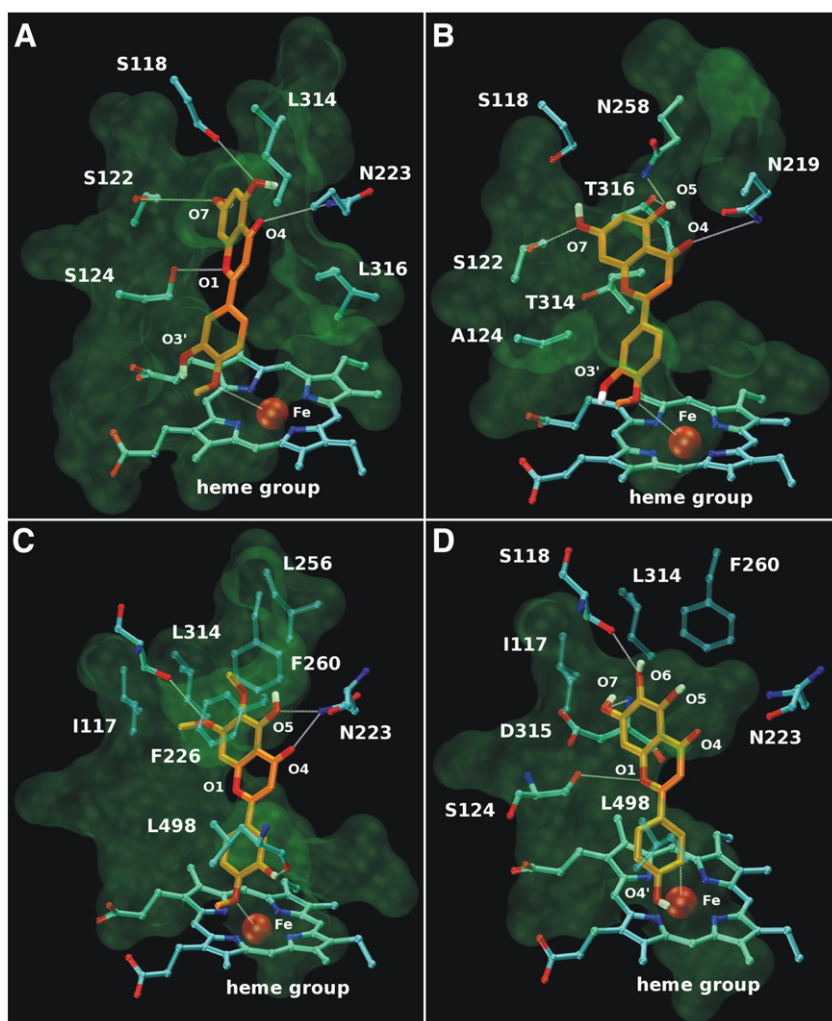
The conversion of eupatorin to cirsiolol was catalyzed to a greater extent by CYP1A1, compared to the 3'-hydroxylation of scutellarein to 6-hydroxy luteolin (Androutsopoulos et al., 2008; Androutsopoulos et al., 2009e). Using the same model of CYP1A1, we have examined the binding mode of eupatorin (Fig. 6C) and scutellarein (Fig. 6D). In the case of eupatorin, the 4'-methoxy group is predicted to approach Fe at  $\sim 5.1$  Å so that the 3'-OH forms a hydrogen-bond with the backbone C O of Leu498. More importantly, the two methyl groups of ring A (positions 6 and 7) exhibit major hydrophobic interactions with Phe226, Phe260, Leu314 and Ile117 (Fig. 6C). CYP1A1–scutellarein model revealed that these hydrophobic contacts are absent due to the presence of the 6- and 7-OH groups (Fig. 6D). In addition, scutellarein was predicted to bind at CYP1A1 without hydrogen-bonding interactions with either Asn223 or Leu498. These observations may support the structural basis of the increased affinity of CYP1A1 for eupatorin 4'-*O*-demethylation with respect to scutellarein 3'-*O*-hydroxylation, which is reflected in the predicted difference in their free energy of binding of  $\sim 1.8$  kcal/mol (unpublished data).

The observations from our molecular modeling analysis, along with further unpublished data from our group, suggest that flavonoids carrying several methoxy groups in the A ring exhibit higher binding affinity for CYP1A1, with respect to their hydroxylated analogues, probably due to the more extensive hydrophobic interactions in the active site. This suggestion is in agreement with previously published studies that have examined metabolism of methoxylated and hydroxylated flavonoids by CYP1 enzymes (Walle & Walle, 2007; Androutsopoulos et al., 2008; Androutsopoulos et al., 2009a,b,d,e unpublished data).

## 5. Bioavailability of dietary flavonoids-CYP1 activation in cancer therapy and prevention

Cytochrome P450 aromatic hydroxylation reactions are generally thought to enhance the bioactivity of their substrates. The differential overexpression of CYP1A1 and CYP1B1 in tumor tissues has been extensively investigated by the Murray group (Murray et al., 1995; Murray et al., 1997; McFadyen et al., 2004). Recently Murray and colleagues have succeeded in the introduction of a CYP1B1 vaccine for the treatment of various types of solid tumors in clinical phase I trials (McFadyen & Murray, 2005; Gribben et al., 2005). In addition, it has been proposed that CYP1B1 and CYP1A1 can be used as targets for chemotherapy by the design of specific pro-drugs that are activated intratumorally to cytotoxic or cytostatic agents, whereas dietary flavonoids





**Fig. 6.** Molecular models of CYP1–flavone complexes. (A) CYP1A1–diosmetin, (B) CYP1B1–diosmetin, (C) CYP1A1–eupatorin and (D) CYP1A1–scutellarein. Atom colors are: orange for the C of substrates, cyan for the C of the CYP1 residues, blue for N, red for O and dark orange for Fe. The green surface represents hydrophobic residues exhibiting contacts with the substrates.

that show substrate specificity for the latter enzymes may be regarded as natural pro-drugs, causing selective inhibition of cancer cell proliferation (Patterson & Murray 2002; McFadyen et al., 2004; McFadyen & Murray, 2005; Androutsopoulos et al., 2009c; Arroo et al., 2009). Piceatannol, the conversion product of resveratrol metabolism by CYP1B1 is a known tyrosine kinase inhibitor, whereas luteolin the conversion product of diosmetin metabolism by CYP1 enzymes is an EGFR and CDK4-cyclin D1 inhibitor. Eupatorin conversion to cirsiolol and two unidentified metabolites causes G2/M arrest of MDA-MB-468 breast cancer cells in vitro, whereas genkwanin metabolism to apigenin further enhances the toxicity of the compound in the latter cell line (Androutsopoulos et al., 2008; Androutsopoulos et al., 2009e). Increased antiproliferative activity of CYP1-mediated flavonoids metabolites as opposed to their parent compounds has been observed in MCF-7 cells for the isoflavone daidzein and the flavone diosmetin (Atherton et al., 2006; Androutsopoulos et al., 2009a). Thus it seems that at least in in vitro cell culture models CYP1–flavonoid activation can prevent further growth of cancer cells by various mechanisms of action. More importantly cellular proliferation of the corresponding non-CYP1 expressing cell lines is not considerably affected.

The metabolic fate, however, of dietary flavonoids in vivo appears to be more complex. Hydroxylated flavonoids are present in very small quantities in their aglycone form in dietary products. In contrast, flavonoid glucosides are more abundantly distributed, in which one or more sugar groups are attached to phenolic hydroxyl groups by a

glycosidic bond. The positions 7, 4' or 3 of the aglycone moiety are usually substituted with a glucose or similar sugar moiety, such as quercetin-4'-O- $\beta$ -glucoside and 3,4'-O- $\beta$ -diglucoside which are present in onion, and rutin or quercetin-3-O-rutinoside and diosmin or diosmetin-7-O-rhamnoside present in citrus fruits. Flavonoid glycosides are absorbed in the small intestine by the sodium dependent glucose transporter (SGLT-1) and pushed back to the lumen by the apical transporter multidrug resistance protein 2 (MDRP2) (Walle, 2004). In some cases flavonoid glucosides were detected in human blood plasma, although the general consensus is that inside the enterocytes they are hydrolyzed by broad specific- $\beta$ -glucosidase enzymes (BS $\beta$ G), to their aglycone (Murota & Terao, 2003). The enzyme LPH lactate phlorizin hydrolase (LPH) located in the brush border of the small intestine, responsible for the hydrolysis of lactose, is also capable of catalyzing hydrolysis of certain glucosides, such as quercetin glucosides to their aglycone (Murota & Terao, 2003). The latter may undergo further conjugation reactions by UDP-glucuronosyltransferase enzymes (UGT) or sulfotransferase enzymes (SULT) before it reaches systemic circulation. A portion of those metabolites may be excreted in the bile and return to the intestinal lumen. In some cases flavonoid aglycones are metabolized to a large extent to secondary byproducts such as CO<sub>2</sub> by colonic microflora (Walle et al., 2001b). Finally, hydrolysis of the flavonoids glycosides is not limited to the colon, as it has been shown by some studies to occur in the oral cavity (Walle, 2004).

It is generally believed that hydroxylated flavonoids, ingested daily, reach very small amounts in their aglycone form in plasma to be considered therapeutically active. Quercetin was found at very low levels in the aglycone form, after oral administration of doses ranging from 8 to 50 mg, whereas glucuronides and sulfates were clearly detectable in plasma (Erlund et al., 2000). However, a similar study from the same group revealed that the peak concentration of the flavonoid naringenin in healthy volunteers ingesting orange juice can be as high as 6  $\mu\text{M}$ , although a very small sample size was used (five women and three men) (Erlund et al., 2001). Furthermore the bioavailability of the flavonoid chrysin was estimated to be 0.003–0.02% after oral administration of 400 mg doses to human volunteers (Walle et al., 2001a). Chrysin was extensively metabolized in the intestine mainly to glucuronic acid and sulfate conjugates (Otake et al., 2002). Studies in CaCo2 cell monolayers and rats have been employed as models for the examination of the bioavailability and the intestinal disposition of dietary flavonoids, in order to avoid the difficulties encountered with human studies (Walle, 2004).

In contrast to the view that flavonoid glucosides are hydrolyzed in the lower part of the intestine to release the corresponding aglycone in the systemic circulation, which was established in the mid 1970s and early 1980s, subsequent experimental investigations in the past twenty years have revealed an extensive network of flavonoid metabolism occurring in the small intestine. It is well established that intestinal metabolism is also important, since glucuronic acid conjugates of flavonoids have been well documented with respect to the molecular site of glucuronidation and the UDP-glucuronosyltransferase isoforms involved (Galijatovic et al., 1999; Boersma et al., 2002; Walle, 2004). It is generally believed that in the intestine sulfation is relatively more important than glucuronidation, whereas the opposite is observed in the liver. Walle and colleagues have pioneered this research area and provided important insight regarding the complexity that underlies intestinal absorption and disposition of dietary flavonoids.

More importantly, recent studies by the Walle group suggest that a certain class of dietary flavonoids, which is found exclusively in the aglycone form, can be regarded as a superior anticancer flavonoid subclass, due to particularly desirable pharmacokinetic properties and chemical stability. Flavonoids which belong to this subclass are the flavones tangeretin, nobiletin and sinensetin which are present in citrus fruits, notably in the skin, and contain five to six methoxy groups. Walle and colleagues have concentrated particularly on 5-,7-dimethoxyflavone and 5-,7-,4'-tri-methoxyflavone, which are the fully methoxylated analogues of chrysin and apigenin and thus allow a direct comparison of the contribution of methoxy and hydroxyl substitutions in the bioavailability and stability of the corresponding aglycones. Studies in liver S9 microsomes, CaCo2 cell culture and in vivo models proved that methoxylation improves the metabolic stability and half-life of flavones containing solely hydroxyl groups (Wen & Walle, 2006; Walle et al., 2007; Walle & Walle, 2007). A plausible explanation is that methoxy substitution protects these compounds from undergoing conjugation reactions in *O*-atoms by UDP-glucuronosyltransferase enzymes. Secondly, methoxylated flavones such as 5-,7-dimethoxyflavone were demonstrated to have lower uptake in oral carcinoma cells and higher release in the basolateral site in CaCo2 cells, thus avoiding extensive intestinal and oral metabolisms as opposed to their hydroxylated counterparts, which suggests that they will possess higher bioavailability (Wen & Walle, 2006; Walle et al., 2007). Moreover, addition of methoxy groups aids to the protection of flavonoids from hepatic phase I metabolism, since CYP3A4 and CYP1A2 show limited specificity towards oxidative metabolism, compared to the extrahepatic CYP1A1 (Walle & Walle, 2007). Results produced in our laboratory further support these findings and CYP1A1 is proven to be the favored catalyst of oxidative metabolism of the fully methoxylated flavones sinensetin, tangeretin and nobiletin as opposed to CYP1A2 and CYP1B1 (Androutsopoulos et al., 2009e; Surichan et al., unpublished data).

Although substantial attention has been paid to the intestinal and hepatic metabolism of flavonoids in cell culture and animal models, proof of principle of the chemopreventive mechanism of action has been shown in vivo, as mentioned previously (Lamartiniere et al., 1995; Birt et al., 1997; Wei et al., 1998; Birt et al., 2001). The main argument presented to counteract these findings is the artificial dose given to laboratory mice to achieve the desired blockage of tumor development, although in some cases inhibition of tumor progression in vivo has been noted for flavonoid concentrations as low as 5  $\mu\text{M}$  (e.g. apigenin) (Birt et al., 1997; Li et al., 1999). As a result, improvement of the pharmacokinetic properties of flavonoids is an important factor which will determine their bioavailability and chemopreventive or chemotherapeutic action. These issues are of increasing alert to the nutraceutical or pharmaceutical industries whereby dietary and synthetic flavonoids are continuously tested for their potential bioactivity. Apart from the fully methoxylated flavonoids present in citrus fruits, the mono-methoxylated flavone diosmetin is supplied as a dietary supplement under the trademark name "Daflon 500" in the US. Daflon 500 is a mixture of diosmin (90%) and a fraction of dietary flavonoids (10%) expressed as hesperidin and is commercially available for the treatment of chronic venous insufficiency, venous ulcers and hemorrhoids (Lyseng-Williamson & Perry, 2003). Diosmin is a glycoside of diosmetin (7-*O*-rhamnoside) and has also been examined for its use in the prevention of colon carcinogenesis (Tanaka et al., 1997). The absorption of diosmin is improved in Daflon 500, by its micronisation to small particles with less than 2  $\mu\text{m}$  diameter (Lyseng-Williamson & Perry, 2003; Paysant et al., 2008). Furthermore some synthetic flavonoids, such as aminoflavone and 2'-nitroflavone, have demonstrated potent in vivo antitumor activity, although such molecules are more active than dietary flavonoids (up to 1000 fold for aminoflavone), and consequently a smaller dose is required for their therapeutic efficacy (Loaiza-Pérez et al., 2004; Cárdenas et al., 2009).

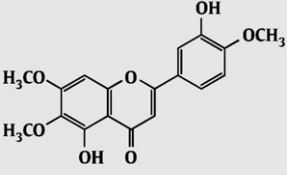
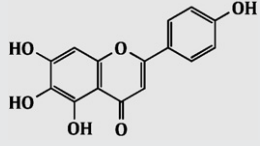
## 6. Future considerations—Potential of methoxylated flavones in chemoprevention

Pro-drug design in cancer therapy was initially focused on the hypoxic environment and the elevated reductase levels present in some tumor types. After the development of several bioreductive agents which were proven to undergo specific activation in hypoxic tumor cells (AQ4N, Mitomycin C, and Tirapazamine), further efforts to pursue this line of research were left unexplored (Phillips, 1998). In some cases, e.g. the bioreductive drug EO9, the major disadvantage encountered was the poor pharmacokinetic profile of the candidate drug (Phillips, 1998). Methoxylated flavonoids are an emerging class of natural products with well tolerated pharmacokinetic properties. These compounds are present in dietary sources that constitute major components of the Western diet e.g. citrus fruits. Diet and chemoprevention must be considered the first choice of treatment, compared to chemotherapy. Recent findings support the notion that dietary methoxylated flavonoids may be regarded as pro-drugs undergoing CYP1- and mainly CYP1A1-mediated metabolism in tumor cells to more pharmacologically active and antiproliferative products. Although much work still remains to illuminate the extensive metabolic pathways of methoxylated flavonoids in vivo, in vitro results presented thus far prove that these natural compounds can be used in cancer therapy or prevention, at doses well within the physiological range. The duality of dietary flavonoids as CYP1-substrates or inhibitors based on their structural features is shown in Table 2.

## 7. Conclusion

Dietary flavonoids are important contributors to cancer prevention, due to their interactions with CYP1 family enzymes. Their chemopreventive effect has been substantially clarified, mainly due to the ability to block the initiation stage of carcinogenesis, which is

**Table 2**  
Biological characteristics of poly-methoxy versus poly-hydroxy flavonoids.

		
Bioavailability	+	–
Effective dose	–	+
CYP1 substrate turnover	+	–
Metabolic conversion	+	–
CYP1-mediated activation	+	–
Net effect	Cancer therapeutic	Chemopreventive

(+) Increased, (–) decreased.

promoted by the latter enzymes. Recent data suggests that in addition to the inhibition of procarcinogen activation, flavonoids exert chemotherapeutic roles by undergoing CYP1-mediated oxidative metabolism to conversion products that inhibit tumor cell growth. Thus there is strong indication that flavonoids act as CYP1 inhibitors and CYP1 substrates. Molecular modeling and site-directed mutagenesis studies have revealed significant hydrogen-bonding and  $\pi$ - $\pi$  stacking interactions with flavonoid molecules and key amino acid residues of CYP1 family enzymes, as means which determine the docking of these compounds to the CYP1 active site. Key features contributing to the orientation to the catalytic site are i) the presence of a methoxy substitution at the 4'-position of the B ring ii) the number of hydroxyl groups on the A and B rings and iii) the hydrophobic interactions of methoxy substitutions on the A ring with non-polar amino acid residues. In addition, extensive investigation of the metabolism of dietary flavonoids in vivo and in vitro models, suggests that methoxylation greatly improves metabolic stability and bioavailability, whereas hydroxylation renders such compounds susceptible to intestinal and hepatic phase I and phase II oxidation and conjugation reactions. Consequently, the function of flavonoids as CYP1 substrates or inhibitors depends on the above mentioned features as prerequisites that determine their entrance into the catalytic site. Although much work still remains to elucidate the in vivo CYP1-mediated activation of dietary flavonoids, the existing knowledge on their substrate/inhibitor interactions with CYP1 enzymes can potentially aid the design of better therapeutics. It is noteworthy that amongst the most prominent cancer therapeutic effects of flavonoids is their function as cyclin dependent kinase (CDK) inhibitors and cyclin dependent kinase inhibitor inducers. The pan-CDK inhibitor flavopyridol is an example of the potential to explore the structural diversity of natural flavonoids and enhance their bioactivity by synthetic analogues. A similar approach would include the design of a flavonoid prodrug that is specifically activated in tumor cells by either CYP1A1 or CYP1B1 to a potent cell cycle inhibitor. The conversion of diosmetin to luteolin and of resveratrol to piceatannol is an example of the contribution of CYP1-activating capacity to the enhancement of the cancer therapeutic effect of flavonoids, since luteolin is a weak CDK4 and EGFR inhibitor and piceatannol a weak tyrosine kinase inhibitor. Future studies should focus on the aromatic hydroxylase activity of CYP1A1 and CYP1B1 on natural and synthetic flavonoids, as means to design chemotherapeutic drugs and the exploration of the flavonoid–CYP1-activating effect in animals and humans, a model that can potentially offer substantial insight into the mechanism of dietary cancer therapy.

## References

- Andres, A., Donovan, S. M., & Kuhlenschmidt, M. S. (2009). Soy isoflavones and virus infections. *J Nutr Biochem* 20, 563–569.
- Androustopoulos, V., Arroo, R. R., Hall, J. F., Surichan, S., & Potter, G. A. (2008). Antiproliferative and cytostatic effects of the natural product eupatorin on MDA-

- MB-468 human breast cancer cells due to CYP1 mediated metabolism. *Breast Cancer Res* 10, R39.
- Androustopoulos, V., Arroo, R. R., Wilsher, N., & Potter, G. A. (2009). Bioactivation of the phytoestrogen diosmetin by CYP1 cytochromes P450. *Cancer Lett* 274, 54–60.
- Androustopoulos, V. P., Li, N., & Arroo, R. R. (2009). The methoxylated flavones eupatorin and cirsiol induce CYP1 enzyme expression in MCF7 cells. *J Nat Prod* 72, 1390–1394.
- Androustopoulos, V. P., Mahale, S., Arroo, R. R., & Potter, G. A. (2009). Anticancer effects of the flavonoid diosmetin on cell cycle progression and proliferation of MDA-MB 468 cells, due to CYP1 activation. *Oncol Rep* 21, 1525–1528.
- Androustopoulos, V. P., Ruparelia, K., Arroo, R. R., Tsatsakis, A. M., & Spandidos, D. A. (2009). CYP1-mediated antiproliferative activity of dietary flavonoids in MDA-MB-468 breast cancer cells. *Toxicology* 264, 162–170.
- Androustopoulos, V. P., Tsatsakis, A. M., & Spandidos, D. A. (2009). Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. *BMC Cancer* 9, 187.
- Arroo, R. R. J., Androustopoulos, V., Beresford, K., Ruparelia, K., Surichan, S., Wilsher, N., et al. (2009). Phytoestrogens as natural prodrugs in cancer prevention: dietary flavonoids. *Phytochem Rev* 8, 375–386.
- Arroo, R. R. J., Androustopoulos, V., Patel, A., Surichan, S., Wilsher, N., & Potter, G. A. (2008). Phytoestrogens as natural prodrugs in cancer prevention: a novel concept. *Phytochem Rev* 7, 431–443.
- Atherton, K. M., Mutch, E., & Ford, D. (2006). Metabolism of the soybean isoflavone daidzein by CYP1A2 and the extra-hepatic CYPs 1A1 and 1B1 affects biological activity. *Biochem Pharmacol* 72, 624–631.
- Birt, D. F., Mitchell, D., Gold, B., Pour, P., & Pinch, H. C. (1997). Inhibition of ultraviolet light skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. *Anticancer Res* 17, 85–91.
- Birt, D. F., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol Ther* 90, 157–177.
- Boersma, M. G., van der Woude, H., Bogaards, J., Boeren, S., Vervoort, J., Cnubben, N. H. P., et al. (2002). Regioselectivity of phase II metabolism of luteolin and quercetin by UDP-glucuronosyl transferases. *Chem Res Toxicol* 15, 662–670.
- Breinholt, V. M., Offord, E. A., Brouwer, C., Nielsen, S. E., Brøsen, K., & Friedberg, T. (2002). In vitro investigation of cytochrome P450-mediated metabolism of dietary flavonoids. *Food Chem Toxicol* 40, 609–616.
- Breinholt, V. M., Rasmussen, S. E., Brøsen, K., & Friedberg, T. H. (2003). In vitro metabolism of genistein and tangeretin by human and murine cytochrome P450s. *Pharmacol Toxicol* 93, 14–22.
- Cárdenas, M. G., Zotta, E., Marder, M., & Roguin, L. P. (2009). In vitro induction of apoptosis and in vivo effects of a flavone nitroderivative in murine mammary adenocarcinoma cells. *Int J Cancer* 125, 222–228.
- Chan, H. Y., & Leung, L. K. (2003). A potential protective mechanism of soya isoflavones against 7, 12-dimethylbenz[a]anthracene tumour initiation. *Br J Nutr* 90, 457–465.
- Chang, T. K. H., Chen, J., & Yeung, E. Y. H. (2006). Effect of Ginkgo biloba extract on procarcinogen-bioactivating human CYP1 enzymes: identification of isorhamnetin, kaempferol, and quercetin as potent inhibitors of CYP1B1. *Toxicol Appl Pharmacol* 213, 18–26.
- Chan, H. Y., Wang, H., & Leung, L. K. (2003). The red clover (*Trifolium pratense*) isoflavone biochanin A modulates the biotransformation pathways of 7, 12-dimethylbenz[a]anthracene. *Br J Nutr* 90, 87–92.
- Chaudhary, A., & Willet, K. L. (2006). Inhibition of human cytochrome CYP1 enzymes by flavonoids of St John's wort. *Toxicology* 217, 194–205.
- Choi, E. J., & Kim, T. (2008). Daidzein modulates induction of hepatic CYP1A1, 1B1 and AhR by 7, 12-dimethylbenz[a]anthracene in mice. *Arch Pharm Res* 31, 1115–1119.
- Chung, H. J., Choi, Y. H., Kim, S. H., & Lee, M. G. (2006). Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous ipriflavone in rats. *J Pharm Pharmacol* 58, 449–457.
- Ciolino, H. P., Daschner, P. J., & Yeh, G. C. (1999). Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochem J* 340, 715–722.
- Ciolino, H. P., & Yeh, G. C. (1999). The flavonoid galangin is an inhibitor of CYP1A1 activity and an agonist/antagonist of the aryl hydrocarbon receptor. *Br J Cancer* 79, 1340–1346.



- Ciolino, H. P., Wang, T. T., & Yeh, G. C. (1998). Diosmin and diosmetin are agonists of the aryl hydrocarbon receptor that differentially affect cytochrome P450 1A1 activity. *Cancer Res* 56, 197–206.
- Dai, R., Zhai, S., Wei, X., Pincus, M. R., Vestal, R. E., & Friedman, F. K. (1998). Inhibition of human cytochrome P450 1A2 by flavones: a molecular modeling study. *J Protein Chem* 17, 643–650.
- Don, M. J., Lewis, D. F., Wang, S. Y., Tsai, M. W., & Ueng, Y. F. (2003). Effect of structural modification on the inhibitory selectivity of rutaecarpine derivatives on human CYP1A1, CYP1A2 and CYP1B1. *Bioorg Med Chem Lett* 13, 2535–2538.
- Doostdar, H., Burke, M. D., & Mayer, R. T. (2000). Bioflavonoids: selective substrates and inhibitors for cytochrome P450 CYP1A and CYP1B1. *Toxicology* 144, 31–38.
- Erlund, I., Kosonen, T., Alftan, G., Mäenpää, J., Perttunen, K., Kenraali, J., et al. (2000). Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *Eur J Clin Pharmacol* 56, 545–553.
- Erlund, I., Meririnne, E., Alftan, G., & Aro, A. (2001). Plasma kinetics and urinary excretion of the flavanones naringenin and hesperidin in humans after ingestion of orange juice and grapefruit juice. *J Nutr* 131, 235–241.
- Fuhr, U., Klittich, K., & Staib, A. H. (1993). Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *Br J Clin Pharm* 35, 431–436.
- Galijatovic, A., Otake, Y., Walle, U. K., & Walle, T. (1999). Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. *Xenobiotica* 29, 1241–1256.
- Gradolatto, A., Canivenc-Lavie, M. C., Basly, J. P., Siess, M. H., & Teyssier, C. (2004). Metabolism of apigenin by rat liver phase I and phase II enzymes and by isolated perfused rat liver. *Drug Metab Dispos* 32, 58–65.
- Gribben, J. G., Ryan, D. P., Boyajian, R., Urban, R. G., Hedley, M. L., Beach, K., et al. (2005). Unexpected association between induction of immunity to the universal tumor antigen CYP1B1 and response to next therapy. *Clin Cancer Res* 12, 4430–4436.
- Hadjokas, N. E., Dai, R., Friedman, F. K., Spence, M. J., Cusack, B. J., Vestal, R. E., et al. (2002). Arginine to lysine 108 substitution in recombinant CYP1A2 abolishes methoxyresorufin metabolism in lymphoblastoid cells. *Br J Pharmacol* 136, 347–352.
- Helsby, N. A., Chipman, J. K., Gescher, A., & Kerr, D. (1998). Inhibition of mouse and human CYP 1A- and 2E1-dependent substrate metabolism by the isoflavonoids genistein and equol. *Food Chem Toxicol* 36, 375–382.
- Hollman, P. C. H., & Katan, M. B. (1998). Bioavailability and health effects of dietary flavonols in man. *Arch Toxicol Suppl* 20, 237–248.
- Hu, M., Krausz, K., Chen, J., Ge, X., Li, J., Gelboin, H. L., et al. (2003). Identification of CYP1A2 as the main isoform for the phase I hydroxylated metabolism of genistein and a prodrug converting enzyme of methylated isoflavones. *Drug Metab Dispos* 31, 924–931.
- Iori, F., da Fonseca, R., Ramos, M. J., & Mezniani, M. C. (2005). Theoretical quantitative structure–activity relationships of flavone ligands interacting with cytochrome P450 1A1 and 1A2 isozymes. *Bioorg Med Chem* 13, 4366–4374.
- Jin, N. Z., Zhu, Y. P., Zhou, J. W., Mao, L., Zhao, R. C., Fang, T. H., et al. (2006). Preventive effects of quercetin against benzo[a]pyrene-induced DNA damages and pulmonary precancerous pathologic changes in mice. *Basic Clin Pharmacol Toxicol* 98, 593–598.
- Kim, H. J., Lee, S. B., Park, S. K., Kim, H. M., Park, Y. I., & Dong, M. S. (2005). Effects of hydroxyl group numbers on the B-ring of 5, 7-dihydroxyflavones on the differential inhibition of human CYP 1A and CYP1B1 enzymes. *Arch Pharm Res* 28, 1114–1121.
- Lamartiniere, C. A., Moore, J., Holland, M., & Barnes, S. (1995). Neonatal genistein chemoprevents mammary cancer. *Proc Soc Biol Med* 208, 120–123.
- Lautraite, S., Musonda, A. C., Doehmer, J., Edwards, G. O., & Chipman, J. K. (2002). Flavonoids inhibit genetic toxicity produced by carcinogens in cells expressing CYP1A2 and CYP1A1. *Mutagenesis* 17, 45–53.
- Leung, H. Y., Wang, Y., Chan, H. Y., & Leung, K. O. (2007). Developing a high-throughput system for the screening of cytochrome P450 1A1-inhibitory polyphenols. *Toxicol in vitro* 21, 996–1002.
- Lewis, D. F. V., Gillam, E. M. J., Everett, S. A., & Shimada, T. (2003). Molecular modeling of human CYP1B1 substrate interactions and investigation of allelic variant effects on metabolism. *Chem Biol Interact* 145, 281–295.
- Lewis, D. F., Lake, B. G., & Dickinson, M. (2004). Quantitative structure–activity relationships within a homologous series of 7-alkoxyresorufins exhibiting activity towards CYP1A and CYP2B enzymes: molecular modeling studies on key members of the resorufin series with CYP2C5-derived models of human CYP1A1, CYP1A2, CYP2B6 and CYP3A4. *Xenobiotica* 34, 501–513.
- Lewis, D. F. V., Lake, B. G., George, S. G., Dickinson, M., Eddershaw, P. J., Tarbit, M. H., et al. (1999). Molecular modeling of CYP1 family enzymes CYP1A1, CYP1A2, CYP1A6 and CYP1B1 based on sequence homology with CYP102. *Toxicology* 139, 53–79.
- Lewis, B. C., Mackenzie, P. I., & Miners, J. O. (2007). Comparative homology modeling of human cytochrome P4501A1 (CYP1A1) and confirmation of residues involved in 7-methoxyresorufin O-deethylation by site-directed mutagenesis and enzyme kinetic analysis. *Arch Biochem Biophys* 468, 58–69.
- Li, D. H., Yee, J. A., McGuire, M. H., Murphy, P. A., & Yan, L. (1999). Soybean isoflavones reduce experimental metastasis in mice. *J Nutr* 129, 1075–1078.
- Liggins, J., Bluck, L. J., Runswick, S., Atkinson, C., Coward, W. A., & Bingham, S. A. (2000). Daidzein and genistein content of fruits and nuts. *J Nutr Biochem* 11, 326–331.
- Liu, J., Erickson, S. S., Sivaneri, M., Besspiata, D., Fisher, C. W., & Szklarz, G. D. (2004). The effect of reciprocal active site mutations in human cytochromes P450 1A1 and 1A2 on alkoxyresorufin metabolism. *Arch Biochem Biophys* 424, 33–43.
- Liu, J., Erickson, S. S., Sivaneri, M., Besspiata, D., Fisher, C. W., & Szklarz, G. D. (2003). Characterization of substrate binding to cytochrome P450 1A1 using molecular modeling and kinetic analyses: case of residue 382. *Drug Metab Dispos* 31, 412–420.
- Loaiza-Pérez, A. I., Kenney, S., Boswell, J., Hollingshead, M., Alley, M. C., Hose, C., et al. (2004). Aryl hydrocarbon receptor activation of an antitumor aminoflavone: basis of selective toxicity for MCF-7 breast tumor cells. *Mol Cancer Ther* 3, 715–725.
- Lyseng-Williamson, K. A., & Perry, C. M. (2003). Micronised purified flavonoid fraction: a review of its use in chronic venous insufficiency, venous ulcers and haemorrhoids. *Drugs* 63, 71–100.
- McFadyen, M. C., Melwin, W. T., & Murray, G. I. (2004). Cytochrome P450 enzymes: novel options for cancer therapeutics. *Mol Cancer Ther* 3, 363–371.
- McFadyen, M. C., & Murray, G. I. (2005). Cytochrome P450 1B1: a novel anticancer therapeutic target. *Future Oncol* 2, 259–263.
- Mitropoulou, T. N., Tzanakakis, G. N., Nikitovic, D., Tsatsakis, A., & Karamanos, N. K. (2002). In vitro effects of genistein on the synthesis and distribution of glycosaminoglycans/proteoglycans by estrogen receptor-positive and -negative human breast cancer epithelial cells. *Anticancer Res* 22, 2841–2846.
- Murota, K., & Terao, J. (2003). Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Arch Biochem Biophys* 417, 12–17.
- Murray, G. I., Taylor, V. E., McKay, J. A., Weaver, R. J., Ewen, S. W., Melvin, W. T., et al. (1995). The immunohistochemical localization of drug-metabolizing enzymes in prostate cancer. *J Pathol* 177, 147–152.
- Murray, G. I., Taylor, M. C., McFadyen, M. C., McKay, J. A., Greenlee, W. F., Burke, M. D., et al. (1997). Tumor-specific expression of cytochrome P450 CYP1B1. *Cancer Res* 57, 3026–3031.
- Nikitovic, D., Tsatsakis, A. M., Karamanos, N. K., & Tzanakakis, G. N. (2003). The effects of genistein on the synthesis and distribution of glycosaminoglycans/proteoglycans by two osteosarcoma cell lines depends on tyrosine kinase and the estrogen receptor density. *Anticancer Res* 23, 459–464.
- Otake, Y., & Walle, T. (2002). Oxidation of the flavonoids galangin and kaempferide by human liver microsomes and CYP1A1, CYP1A2, and CYP2C9. *Drug Metab Dispos* 30, 103–105.
- Otake, Y., Hsieh, F., & Walle, T. (2002). Glucuronidation versus oxidation of the flavonoid galangin by human liver microsomes and hepatocytes. *Drug Metab Dispos* 30, 576–581.
- Pastrakuljic, A., Tang, B. K., Roberts, E. A., & Kalow, W. (1997). Distinction of CYP1A1 and CYP1A2 activity by selective inhibition using fluvoxamine and isosafrole. *Biochem Pharmacol* 53, 531–538.
- Patterson, L. H., & Murray, G. I. (2002). Tumor cytochrome P450 and drug activation. *Curr Pharm Des* 15, 1335–1347.
- Paysant, J., Sansilvestri-Morel, P., Bouskela, E., & Verbeuren, T. J. (2008). Different flavonoids present in the micronized purified flavonoid fraction (Daflon 500 mg) contribute to its anti-hyperpermeability effect in the hamster cheek pouch microcirculation. *Int Angiol* 27, 81–85.
- Peng, W. X., Li, H. D., & Zhou, H. H. (2003). Effect of daidzein on CYP1A2 activity and pharmacokinetics of theophylline in healthy volunteers. *Eur J Clin Pharmacol* 59, 237–241.
- Peterson, S., Lampe, J. W., Bammler, T. K., Gross-Steinmeyer, K., & Eaton, D. L. (2006). Apiaecous vegetable constituents inhibit human cytochrome P-450 1A2 (hCYP1A2) activity and hCYP1A2-mediated mutagenicity of aflatoxin B1. *Food Chem Toxicol* 44, 1474–1484.
- Phillips, R. M. (1998). Prospects for bioreductive drug development. *Expert Opin Investig Drugs* 6, 905–928.
- Potter, G. A., Patterson, L. H., Wanogho, E., Perry, P. J., Butler, P. C., Ijac, T., et al. (2002). The cancer preventative agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. *Br J Cancer* 86, 774–778.
- Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., & Kohli, K. (2009). Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflamm Allergy Drug Targets* 8, 229–235.
- Roberts, D. W., Doerge, D. R., Churchwell, M. I., Gamboa da Costa, G., Marques, M. M., & Tolleson, W. H. (2004). Inhibition of extrahepatic human cytochrome P450 1A1 and 1B1 by metabolism of isoflavones found in *Trifolium pretense* (red clover). *J Agric Food Chem* 52, 6623–6632.
- Sansen, S., Yano, J. K., Reynald, R. L., Schoch, G. A., Griffin, K. J., Stout, C. D., et al. (2007). Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2. *J Biol Chem* 282, 14348–14355.
- Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of dietary polyphenols. *J Nutr* 130, 2073S–2085S.
- Schwarz, D., & Roots, I. (2003). In vitro assessment of inhibition by natural polyphenols of metabolic activation of procarcinogens by human CYP1A1. *Biochem Biophys Res Commun* 303, 902–907.
- Schwarz, D., Kisselev, P., & Roots, I. (2003). St John's wort extracts and some of their constituents potentially inhibit ultimate carcinogen formation from benzo[a]pyrene-7, 8-dihydrodiol by human CYP1A1. *Cancer Res* 63, 8062–8068.
- Schwarz, D., Kisselev, P., & Roots, I. (2005). CYP1A1 genotype-selective inhibition of benzo[a]pyrene activation by quercetin. *Eur J Cancer* 41, 151–158.
- Shimada, T., & Fujii-Kuriyama, Y. (2004). Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer Sci* 95, 1–6.
- Siess, M. H., Leclerc, J., Canivenc-Lavie, M. C., Rat, P., & Suschetet, M. (1995). Heterogeneous effects of natural flavonoids on monooxygenase activities in human and rat liver microsomes. *Toxicol Appl Pharmacol* 130, 73–78.
- Silva, I. D., Rodrigues, A. S., Gaspar, J., Maia, R., Laires, A., & Rueff, J. (1997). Involvement of rat cytochrome 1A1 in the biotransformation of kaempferol to quercetin: relevance to the genotoxicity of kaempferol. *Mutagenesis* 12, 383–390.
- Tanaka, T., Makita, H., Kawabata, K., Mori, H., Kakumoto, M., Satoh, K., et al. (1997). Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally occurring flavonoids diosmin and hesperidin. *Carcinogenesis* 18, 957–965.
- Vaouzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., & Spencer, J. P. (2008). The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr* 3, 115–126.
- Walle, T. (2004). Absorption and metabolism of flavonoids. *Free Rad Biol Med* 36, 829–837.



- Walle, U. K., & Walle, T. (2007). Bioavailable flavonoids: cytochrome P450-mediated metabolism of methoxyflavones. *Drug Metab Dispos* 35, 1985–1989.
- Walle, T., Otake, Y., Brubaker, J. A., Walle, U. K., & Halushka, P. V. (2001). Disposition and metabolism of the flavonoid chrysin in normal volunteers. *Br J Clin Pharmacol* 51, 143–146.
- Walle, T., Walle, U. K., & Halushka, P. V. (2001). Carbon dioxide is the major metabolite of quercetin in humans. *J Nutr* 131, 2648–2652.
- Walle, T., Ta, N., Kawamori, T., Wen, X., Tsuji, P. A., & Walle, U. K. (2007). Cancer chemopreventive properties of orally bioavailable flavonoids—methylated versus unmethylated flavones. *Biochem Pharmacol* 73, 1288–1296.
- Wei, H. C., Bowen, R., Zhang, X. S., & Lebowitz, M. (1998). Isoflavone genistein inhibits the initiation and promotion of two-stage skin carcinogenesis in mice. *Carcinogenesis* 19, 1509–1514.
- Wen, X., & Walle, T. (2005). Preferential induction of CYP1B1 by benzo[a]pyrene in human oral epithelial cells: impact on DNA adduct formation and prevention by polyphenols. *Carcinogenesis* 26, 1774–1781.
- Wen, X., & Walle, T. (2006). Methylated flavonoids have greatly improved intestinal absorption and metabolic stability. *Drug Metab Dispos* 34, 1786–1792.
- Wen, X., Walle, U. K., & Walle, T. (2005). 5, 7-Dimethoxyflavone downregulates CYP1A1 expression and benzo[a]pyrene-induced DNA binding in Hep G2 cells. *Carcinogenesis* 26, 803–809.
- Williams, P. A., Cosme, J., Ward, A., Angove, H. C., Matak Vinkovic, D., et al. (2003). Crystal structure of human cytochrome P450 2C9 with bound warfarin. *Nature* 424, 464–468.
- Zhai, S., Dai, R., Friedman, F. K., & Vestal, R. E. (1998). Comparative inhibition of human cytochrome P450 1A1 and 1A2 by flavonoids. *Drug Metab Dispos* 26, 989–992.
- Zhai, S., Dai, R., Wei, X., Friedman, F. K., & Vestal, R. E. (1998). Inhibition of methoxyresorufin demethylase activity by flavonoids in human liver microsomes. *Life Sci* 63, 119–123.