1521-009X/45/1/49–55$25.00
Drug Metabolism and Disposition

http://dx.doi.org/10.1124/dmd.116.073213
Drug Metab Dispos 45:49–55, January 2017

Curcumin, Piperine, and Capsaicin: A Comparative Study of Spice-Mediated Inhibition of Human Cytochrome P450 Isozyme Activities

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Received August 24, 2016; accepted November 3, 2016

ABSTRACT

Inhibition of cytochrome P450 (P450) enzymes (CYP) has been shown to lower the metabolism of drugs that are P450 substrates and to consequently alter their pharmacokinetic profiles. Curcumin (CUR), piperine (PIP), and capsaicin (CAP) are spice components (SC) that inhibit the activities of a range of P450 enzymes, but the selection of which SC to be prioritized for further development as an adjuvant will depend on the ranking order of the inhibitory potential of the SCs on specific P450 isozymes. We used common human recombinant enzyme platforms to provide a comparative evaluation of the inhibitory activities of CUR, PIP, and CAP on the principal drug-metabolizing P450 enzymes. SC-mediated inhibition of CYP3A4 was found to rank in the order of CAP (IC_{50} 1.84 ± 0.71 μM) ~ PIP (2.12 ± 0.45 μM) > CUR (11.93 ± 3.49 μM), while CYP2C9 inhibition was in the order of CAP (11.95 ± 4.24 μM) ~ CUR (14.58 ± 4.57 μM) > PIP (89.62 ± 9.17 μM). CAP and PIP were significantly more potent inhibitors of CYP1A2 (IC_{50} 2.14 ± 0.22 μM and 14.19 ± 4.15 μM, respectively) than CUR (IC_{50} > 100 μM), while all three SCs exhibited weak activity toward CYP2D6 (IC_{50} 95.42 ± 12.09 μM for CUR, 99.99 ± 5.88 μM for CAP, and 110.40 ± 3.23 μM for PIP). Of the three SCs, CAP thus has the strongest potential for further development into an inhibitor of multiple CYPs for use in the clinic. Data from this study are also useful for managing potential drug–SC interactions.

Introduction

Coadministration of an agent that inhibits or induces the cytochrome P450 (P450) phase I drug-metabolizing enzyme systems is an important cause of adverse events and therapeutic failures for drugs with a narrow therapeutic index (Pea and Furlanut, 2001; Hemaiswarya and Doble, 2006). However, where the P450-modulating agent is intentionally coadministered at a titrated dose, there is potential for it to nullify the unacceptably low or variable bioavailability of a drug that undergoes extensive first pass metabolism. It is this potential that drives the development and characterization of a plethora of P450-modulating agents as reported in the literature. To be clinically useful, however, the agent has to have a proven safety profile for human consumption, and on this basis, food-derived P450-modulating agents, such as the spice components (SCs) curcumin (CUR), piperine (PIP), and capsaicin (CAP) (Fig. 1), can enjoy higher acceptance than novel compounds.

CUR, a polyphenolic component of turmeric (*Curcuma longa*), has promising therapeutic potential as an anticancer (Ide et al., 2010), antioxidant (Hismiogullari et al., 2015), and anti-inflammatory agent (Kaur et al., 2015). CUR is generally considered to be a safe compound, with oral doses as high as 8 g/day having been administered to humans without overt side effects (Cheng et al., 2001). At these doses, peroral CUR has been shown to increase drug bioavailability by attenuating intestinal CYP3A expression (Zhang et al., 2007; Zhang and Lim, 2008). CUR is also reported to be a potent inhibitor of CYP1A1, CYP1A2, and CYP2B1 activities in rat liver microsomes (Thapliyal and Maru, 2001). In vivo, CUR administered orally to Sprague-Dawley rats at 60 mg/kg once daily over 4 days resulted in the down-regulation of intestinal CYP3A to levels significant enough to affect the pharmacokinetic parameters of coadministered midazolam (Zhang et al., 2007). The enhancement of docetaxel bioavailability in the rat model by CUR, coadministered at 100 mg/kg for 4 consecutive days, has also been attributed in part to an inhibition of CYP3A activity (Yan et al., 2012).

PIP, the active component of black pepper (*Piper nigrum* Linn) and long pepper (*Piper longum* Linn), has also been shown, although in a smaller number of studies, to inhibit P450 enzymes. Unlike CUR, PIP is a noncompetitive inhibitor of CYP3A4 and a less potent inhibitor of CYP1A2 and CYP2D6 (Volak et al., 2008). In vivo, PIP coadministered orally to C.B17/lcr-scid mice at 100 mg/kg (Makhov et al., 2012) and to Swiss albino mice at 10 mg/kg (Venkatesh et al., 2011) has been found to increase, respectively, the bioavailability of docetaxel and ibuprofen, two drugs with extensive first-pass metabolic profiles.

CAP, the pungent ingredient of chilli peppers, is another SC shown to modulate P450 isozymes (Takanohashi et al., 2010; Han et al., 2012; Kato et al., 2012). CAP at dietary concentrations may not be a P450 inhibitor, but at concentrations higher than 2.0 μM, it has been shown to inhibit CYP2D6 and CYP1A2 activities sufficiently to impact drug metabolism (Babbar et al., 2010). CAP may also modify the pharmacokinetic profiles of drugs that are CYP3A substrates (Hirotani et al.,

This study was supported by grants from the University of Western Australia, Australia. Suhaili Shamsi was the recipient of a graduate scholarship from the Ministry of Higher Education, Malaysia.
dx.doi.org/10.1124/dmd.116.073213.

ABBREVIATIONS: BOMCC, 7-benzyloxymethyloxy-3-cyanocoumarin; CAP, capsaicin; CUR, curcumin; CYP, cytochrome P450 enzyme; DMSO, dimethylsulfoxide; EOMCC, 7-ethylmethyloxy-3-cyanocoumarin; GI, gastrointestinal; HLM, human liver microsomes; KCZ, ketoconazole; NPV, α-napthoflavone; P450, cytochrome P450; PIP, piperine; QDN, quinidine; SC, spice components; SPZ, sulfaphenazole.

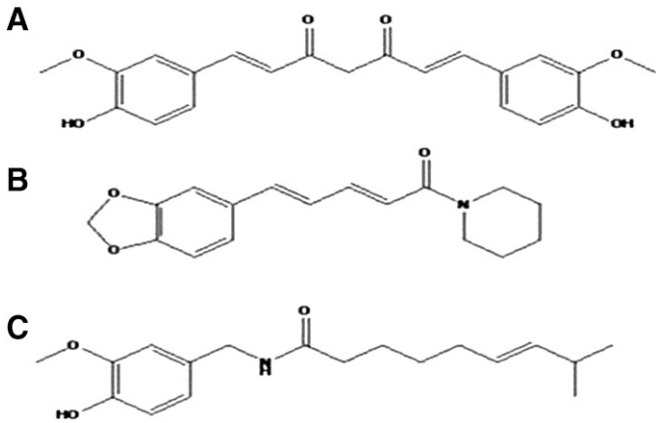

Fig. 1. Chemical structure of (A) curcumin (CUR), (B) piperine (PIP), and (C) capsaicin (CAP).

2007; Zhai et al., 2013). In rats, the administration of CAP at a dose of 3 mg/kg/day over 7 days was found to increase the plasma concentration of cyclosporine, and this was attributed to the lower expression of hepatic and intestinal CYP3A mRNA and protein levels (Zhai et al., 2013).

Collectively, therefore, there is adequate evidence showing the SCs to be P450 inhibitors capable of interacting with drugs. The clinical potentials of PIP (Volak et al., 2008; Wang et al., 2013), CUR (Zhang et al., 2007; Volak et al., 2008), and CAP (Zhang et al., 2012; Zhai et al., 2013) as P450 inhibitors have also been examined. The three SCs are interesting because they share similarities in chemical structures (Fig. 1) and are complementary in taste, often coexisting in many Asian cuisines. On this basis, we hypothesized that CUR, PIP, and CAP would exhibit comparable inhibitory activities against the major drug-metabolizing P450 isozymes.

However, there is as yet no published study that directly compares the actions of CUR, PIP, and CAP on these enzymes. Data collated from reports using different methodologies do not allow for quantitative comparisons, and enzymes derived from human liver microsomes (HLM) and hepatocytes, particularly when the microsomes and hepatocytes are not from the same donors, would not allow for valid data comparison across laboratories or time frames. This then makes it difficult to have agreement on the relative effectiveness of the SCs that will inform clinical decisions on which SC is optimal for when a specific P450 activity is to be regulated.

The present study addresses this information gap by comparing the P450-inhibitory effectiveness of CUR, PIP, and CAP using common enzyme platforms, and ascertaining whether any of the SCs was an effective inhibitor of all the tested enzymes. The enzymes studied were CYP1A2, CYP2C9, CYP2D6, and CYP3A4, which collectively account for the metabolism and disposition of about 70% of therapeutic drugs available in the market (Williams et al., 2004). Confounding factors inherently present in biologic tissues were minimized by using purified human recombinant enzymes supplied by the same manufacturer to generate the SC-mediated CYP-modulating data for comparisons. Our results showed that CAP exhibited the highest CYP-inhibitory potential and should therefore be prioritized for further development into a pharmaceutic P450 inhibitor.

Materials and Methods

Materials. Analytic-grade dimethylsulfoxide (DMSO) and acetonitrile were purchased from Ajax Finechem (Taren Point, Australia). PIP (≥97%) was obtained from SAFC Global (St. Louis, MO). We purchased α-naphthoflavone, ketoconazole (≥98%), sulfaphenazole (≥99%), CUR (≥94), CAP (≥95%), and quinidine from Sigma Chemicals (Castle Hill, Australia). The P450 inhibition assays were conducted using the Vivid CYP1A2, CYP3A4, CYP2C9, and CYP2D6 Blue Screening Kits purchased from Invitrogen (Carlsbad, CA). The kits contained microsomes from baculovirus-infected cells coexpressing human CYP1A2, CYP2D6, CYP2C9, or CYP3A4, together with NADPH-cytochrome P450 reductase (P450 baculosomes) and the NADPH regeneration system containing glucose-6- phosphate and glucose-6-phosphate dehydrogenase. The kits also contained 7-benzyloxymethyloxy-3-cyanocoumarin (BOMCC) as the substrate for CYP2C9 and CYP3A4, and 7-ethylmethyloxy-3-cyanocoumarin (EOMCC) as the substrate for CYP1A2 and CYP2D6. Deionized water was used throughout.

Test and Control Samples. Test samples with the SC concentration range of 0 to 120 μM, the upper concentration limited by the SC's solubility in the medium, were prepared by dissolving the SC in a pH 8.0 potassium phosphate buffer (reaction buffer supplied by the manufacturer) supplemented with 0.5% DMSO. Positive control samples consisted of the manufacturer-specified inhibitor dissolved in the same medium; α-naphthoflavone (NPV, 1.7 and 10 μM) for CYP1A2, sulfaphenazole (SPZ, 20 and 30 μM) for CYP2C9, quinidine (QDN, 0.6 and 10 μM) for CYP2D6, and ketoconazole (KCZ, 5 and 10 μM) for CYP3A4. Blank medium (0.5% DMSO in reaction buffer) constituted the negative control sample to account for any effects of DMSO on P450 inhibition (Trubetskoy et al., 2005b).

P450 Inhibition Assays. Assays were conducted as triplicate independent experiments for each SC according to the Vivid assay protocols provided by the manufacturer (Invitrogen). All enzyme reactions were conducted under conditions shown to be linear with respect to incubation time and amounts of enzyme and substrate (Trubetskoy et al., 2005b). The inhibition assays were performed in 96-well plates. The final enzyme and substrate concentrations are given in Table 1.

The integrity of the recombinant enzymes was confirmed using the respective substrates with and without coincubation with the specified positive control. A negative control sample was included in every experiment. Test and control samples were dispensed into 96-well plates, together with 50 μl of the Master Pre-Mix solution. The background fluorescence was measured (λ_{ex} 405 nm, λ_{em} 460 nm; FLUOstar Optima; BMG Labtech, Victoria, Australia) after incubation for 10 minutes at 37°C (Contherm Scientific, Lower Hutt, New Zealand).

The enzyme reaction was initiated by adding 10 μl of Pre-Mix solution, and sufficient reaction buffer to give a final volume of 100 μl. The reaction proceeded in the dark for 20 minutes at 37°C before it was quenched by adding 10 μl of the Stop Reagent. The metabolites produced were quantified by fluorescence measurements. The plate reader was calibrated with the four fluorescent metabolites, the respective standard curves constructed over a concentration range of 0 to 2500 nM using serially diluted standards provided by the kit manufacturer.

Enzyme activity was calculated based on relative fluorescence unit (rfu) using the following equation:

$$\%\ \text{Enzyme activity} = \frac{\text{rfu in test samples or positive control}}{\text{rfu in blank}} \times 100$$

Data Analyses. The IC_{50} value for each SC–P450 pair was determined from nonlinear regression analysis of residual enzyme activity versus SC concentration. Data obtained from triplicate independent experiments are expressed as mean ± standard deviation (S.D.). Enzyme activities at two different inhibitor concentrations (Fig. 2) were compared using the paired t test. Data for each SC-P450 interaction (Figs. 3, 4, and 5) and the IC_{50} values of the three SCs for each P450 isozyme were analyzed using one-way analysis of variance (ANOVA) with Tukey's test applied post hoc for paired comparison of means. $P \leq 0.05$ was considered statistically significance (SPSS 16.0; SPSS Inc., Chicago, IL).

TABLE 1

General reaction conditions for the Vivid P450 inhibition assays

P450 Baculosomes	Vivid Substrate	CYP450 (nM)	Vivid Substrate (μM)
CYP1A2	EOMCC	5	3
CYP2C9	BOMCC	10	10
CYP2D6	EOMCC	10	10
CYP3A4	BOMCC	5	10

BOMCC, 7-benzyloxymethyloxy-3-cyanocoumarin; EOMCC, 7-ethylmethyloxy-3-cyanocoumarin. All assays were performed with 3.33 mM glucose-6-phosphate, 0.3 U/ml glucose-6-phosphate dehydrogenase, and 30 μM NADP+ in potassium phosphate buffer, pH 8.0.

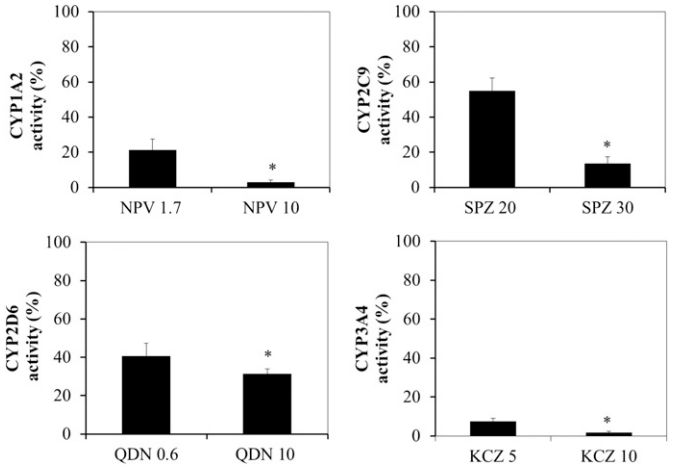

Fig. 2. Concentration-dependent inhibition of human recombinant CYP1A2, CYP2C9, CYP2D6, and CYP3A4 isozymes by naphthoflavone (NPV, 1.7 and 10 μM), sulfaphenazole (SPZ, 20 and 30 μM), quinidine (QDN, 0.6 and 10 μM), and ketoconazole (KCZ, 5 and 10 μM), respectively. Data represent the mean ± S.D. (n = 3) of residual enzyme activity expressed as the percentage of enzyme activity in the absence of inhibitor. *$P < 0.05$, statistically different inhibitory activities between the two concentrations of an inhibitor.

Results

Integrity of P450 Enzyme Systems. All four recombinant human P450 isozymes employed in this study were functional, and the resultant metabolites were readily quantifiable by fluorescence measurements. Calibration plots for each metabolite yielded linear lines with $R^2 \geq 0.95$ over the concentration range of 0 to 2500 nM. P450 activity was significantly reduced but not negated by coincubation of the specified substrate and inhibitor at the concentrations recommended by the manufacturer (Fig. 2). The extent of P450 inhibition was dependent on the inhibitor concentration, with greater suppression of the enzyme activities noted at higher inhibitor concentrations. The collective data showed that the integrity of all four recombinant enzyme systems was intact and that they could be used to compare the P450-modulating activity of the SCs.

CUR-Mediated P450 Inhibition. CUR was an inhibitor of all four recombinant enzymes, its effectiveness was dependent on its concentration and the type of P450 isozyme employed (Fig. 3). Of the four P450 isozymes, CUR was most effective at inhibiting CYP3A4, almost obliterating the enzyme function at concentrations ≥60 μM. It was less effective against CYP1A2, where residual enzyme levels ≥40% were observed even at 120 μM of CUR (Fig. 3). Compared with the established P450 inhibitors, CUR produced comparable inhibitions at a lower concentration than SPZ (15 μM versus 20 μM) for CYP2C9, and at higher concentrations than KCZ (30 μM versus 5 μM) and QDN (120 μM versus 0.6 μM) for CYP3A4 and CYP2D6, respectively. It was possible by applying higher concentrations of CUR to inhibit CYP2C9 and CYP3A4 to levels lower than those induced by the manufacturer-recommended inhibitors (Fig. 3).

PIP-Mediated P450 Inhibition. PIP also exhibited inhibitory activity against all four recombinant enzymes in a manner that was influenced by its concentration and the P450 isoenzyme employed (Fig. 4). Like CUR, PIP was most potent against CYP3A4; however, while it was able to halve the enzyme activity at a very low concentration of 2 μM, PIP produced comparable CYP3A4 inhibition to KCZ at a higher concentration (30 μM versus 5 μM), and the CYP3A4 function could not be lowered further even when PIP was increased to 120 μM. PIP was also able to produce comparable enzyme inhibitions as the manufacturer-recommended inhibitors. However, this was achieved at higher concentrations than SPZ (30 μM versus 20 μM) and NPV (30 μM versus 1.7 μM) for CYP2C9 and CYP1A2, respectively, and the PIP-mediated inhibitory action on these two P450 isozymes showed leveling effects at higher concentrations. PIP was least effective against CYP2D6, where the residual enzyme activity at 120 μM of PIP was significantly higher than that seen with 0.6 μM of QDN.

CAP-Mediated P450 Inhibition. CAP was an effective inhibitor of all four recombinant isozymes, lowering the enzyme activities to match those seen with the established inhibitors (Fig. 5), although CAP achieved this at relatively higher concentrations. The exception was

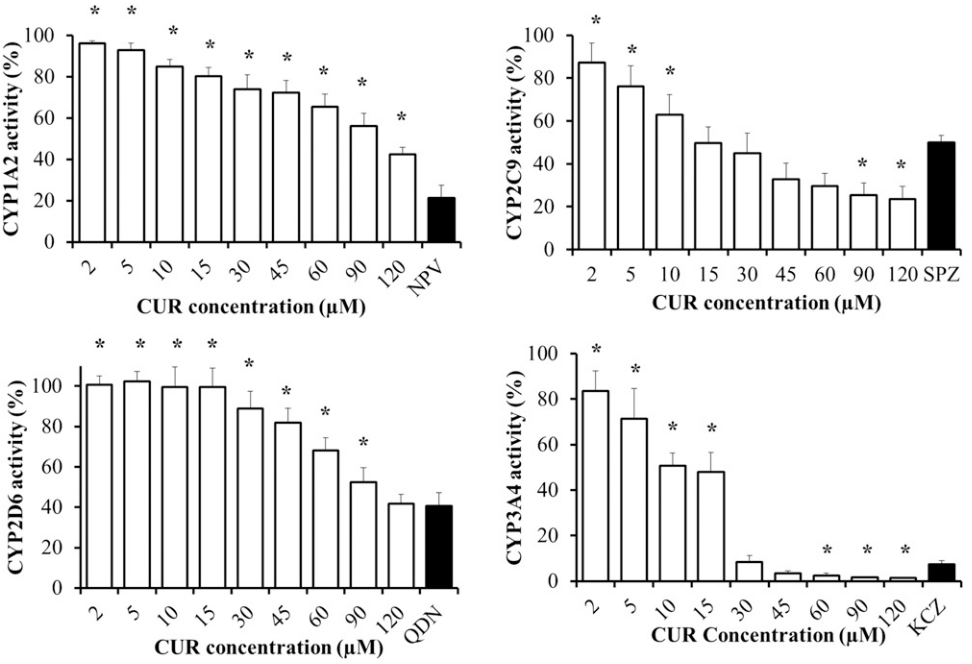

Fig. 3. CUR-mediated inhibition of CYP1A2, CYP2C9, CYP2D6, and CYP3A4 as compared with naphthoflavone (NPV, 1.7 μM), sulfaphenazole (SPZ, 20 μM), quinidine (QDN, 0.6 μM), and ketoconazole (KCZ, 5 μM), respectively. Data represent the mean ± S.D. (n = 3) of enzyme activity expressed as the percentage of enzyme activity in the absence of inhibitor. *$P < 0.05$, statistically different inhibitory activity compared with the respective manufacturer-recommended inhibitor.

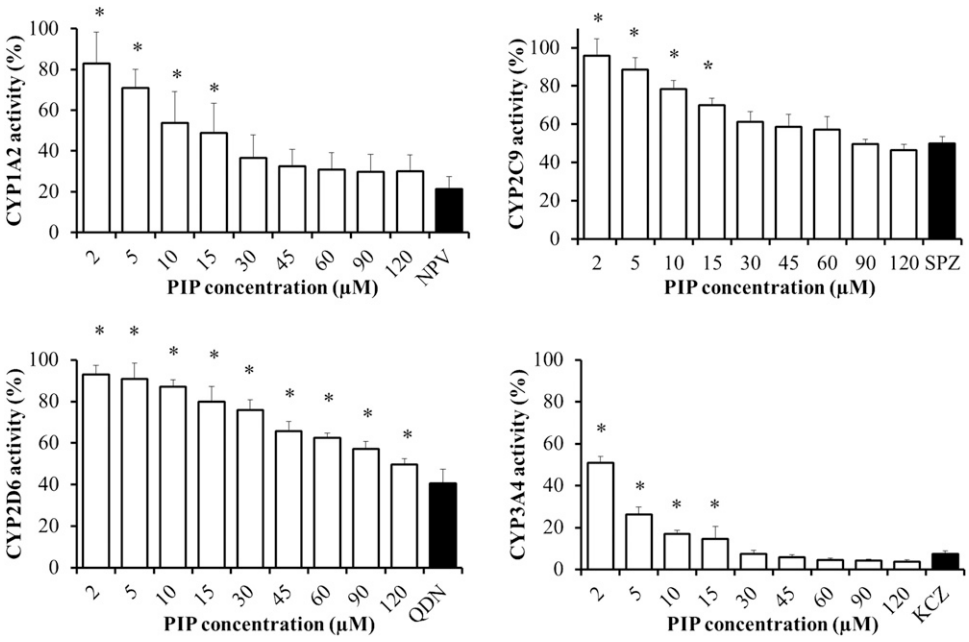

Fig. 4. PIP-mediated inhibition of CYP1A2, CYP2C9, CYP2D6, and CYP3A4 as compared with α-napthoflavone (NPV, 1.7 μM), sulfaphenazole (SPZ, 20 μM), quinidine (QDN, 0.6 μM), and ketoconazole (KCZ, 5 μM), respectively. Data represent the mean ± S.D. (n = 3) of enzyme activity expressed as the percentage of enzyme activity in the absence of inhibitor. *$P < 0.05$, statistically different inhibitory activity compared with the respective manufacturer-recommended inhibitor.

CYP2C9, where the inhibitory action of CAP at 10 μM was comparable to that of SPZ at 20 μM, and higher levels of enzyme inhibition were noted on increasing CAP to ≥60 μM. With CYP3A4, CAP exhibited an inhibitory profile that has characteristics seen with PIP (strong inhibition at low concentrations) and CUR (concentration-dependent inhibition even at higher concentrations).

Comparative SC-Mediated P450 Inhibition. The IC_{50} values determined from the nonlinear regression analysis of residual enzyme activity versus SC concentration were in the μM range for all three SCs (Table 2). Based on the relative IC_{50} values, the ranking order of inhibitory potential against CYP1A2 was CAP > PIP > CUR, while the three SCs were equally weak at inhibiting CYP2D6. CAP and PIP were comparable in their inhibitory potential against CYP3A4, and this was >5-fold higher than that seen with CUR. In contrast, CUR was comparable in inhibitory potential to CAP against CYP2C9, the inhibitory potential being >6-fold higher than that observed with PIP.

The IC_{50} values for the manufacturer-recommended inhibitors have been published for a variety of enzyme platforms employed (Table 2). When compared against these IC_{50} values, CUR, PIP, and CAP were relatively weaker P450 inhibitors, although CAP has the potential to match the potency of SPZ in inhibiting CYP2C9.

Discussion

This study evaluated the comparative capability of CUR, PIP, and CAP to inhibit four major human P450 isozymes responsible for drug bioavailability and disposition. Studies investigating SC-mediated inhibition of P450 functional activities are not new. However, as shown

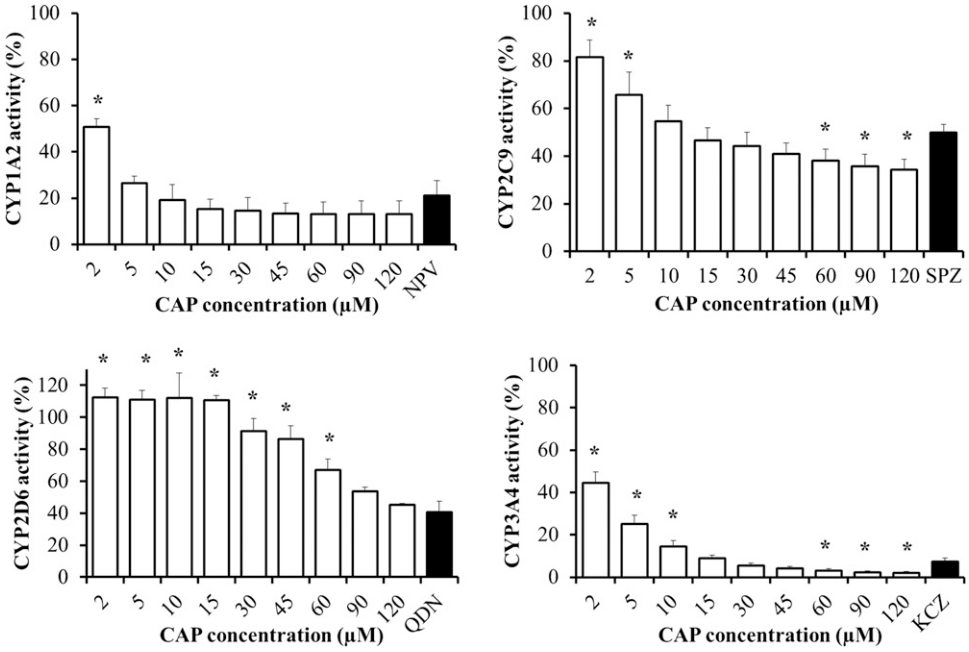

Fig. 5. CAP-mediated inhibition of CYP1A2, CYP2C9, CYP2D6, and CYP3A4 as compared with α-napthoflavone (NPV, 1.7 μM), sulfaphenazole (SPZ, 20 μM), quinidine (QDN, 0.6 μM), and ketoconazole (KCZ, 5 μM), respectively. Data represent the mean ± S.D. (n = 3) of enzyme activity expressed as the percentage of enzyme activity in the absence of inhibitor. *$P < 0.05$, statistically different inhibitory activity compared with the respective manufacturer-recommended inhibitor.

TABLE 2

IC_{50} values (μM) for CUR, PIP, CAP and the manufacturer-recommended inhibitors against CYP1A2, CYP2C9, CYP2D6, and CYP3A4 isozymes activity

Data represent mean ± S.D. (n = 3).

P450 Isozyme	IC_{50} (μM)			Published IC_{50} (μM) for Inhibitor
	CUR	PIP	CAP	
CYP1A2	101.73 ± 10.07[a]	14.19 ± 4.15[b]	2.14 ± 0.22[a,b]	0.26 for NPV (Nayadu et al., 2013)[c]
CYP2C9	14.58 ± 4.57[a]	89.62 ± 9.17[b]	11.95 ± 4.24[a]	10.00 for SPZ (Zhang et al., 2008)[d]
CYP2D6	95.42 ± 12.09	110.40 ± 3.23	99.99 ± 5.88	0.50 for QDN (Kong et al., 2011)[c]
CYP3A4	11.93 ± 3.49[a]	2.12 ± 0.45[b]	1.84 ± 0.71[b]	0.54 for KCZ (Trubetskoy et al., 2005a)[e]

[a] $P < 0.05$, significant difference to PIP.
[b] $P < 0.05$, significant difference to CUR.
[c] Recombinant human isozyme.
[d] Human liver microsomes.
[e] DPX-2 cell line.

in Table 3, the published IC_{50} data for a SC-P450 combination typically span a broad range of values, depending on the SC grade, enzyme source, substrate, inhibitor, and assay method employed. This then makes it difficult to use the published IC_{50} values to accurately assess the comparative P450-inhibitory potency of the SC.

In this study we employed a common high-throughput P450 enzyme assay kit to evaluate the three SCs against a specific P450 activity. The recombinant enzyme system may be more costly and less representative of physiologic conditions, but it is a more consistent platform that avoids the wide variability in enzyme expression and activity normally encountered in HLM (Snawder and Lipscomb, 2000) and hepatocytes (Rodriguez-Antona et al., 2002; Westerink and Schoonen, 2007). Also, the enzyme systems are highly specific and relatively stable, with no significant loss in activity noted after 7 hours at room temperature (Trubetskoy et al., 2005b).

Fluorescent high-throughput screening methods employ fluorescent P450 substrates that are efficiently metabolized by specific P450 isozymes to yield a product with altered fluorescent properties, usually increased fluorescent intensity (Trubetskoy et al., 2005b). The assay requires only low reactant volume to produce high signal-to-background ratio, which allows multiple SCs to be evaluated using the same batch of recombinant P450 enzyme. On the basis of such direct comparisons, CAP was shown to be the most promising inhibitor of multiple P450 isozymes, and its effects on drug bioavailability should be further examined in clinical trials.

CAP not only exhibited the lowest mean IC_{50} values against CYP3A4, CYP1A2, and CYP2C9, but it was also the only SC to exhibit P450-inhibiting activity equivalent to all four positive controls at concentrations $\leq$90 μM. Despite the recombinant enzyme systems being less physiologically relevant, there is general consensus between our data and the published IC_{50} values on the rank order of P450 inhibition by CUR and PIP, with CUR having greater activity against CYP2C9 and CYP3A4 than against CYP2D6 and CYP1A2 (Appiah-Opong et al., 2007; Volak et al., 2008; Bamba et al., 2011), and PIP exhibiting stronger inhibition of CYP3A4 and CYP1A2 (Volak et al., 2008). CUR has been reported to demonstrate moderate inhibitory action on CYP2C9, 2C19, and 2B6 activities in the HLM (Volak et al., 2008), and CYP3A4, 2C9, 2D6, and 1A2-mediated metabolism of fluorogenic probe substrates in recombinant enzyme systems (Appiah-Opong et al., 2007).

The rank order for CAP-mediated P450 inhibition was less consistent with the published literature. In the present study, CAP was more active against the CYP3A4 and CYP1A2, while Babbar et al. (2010) reported stronger activity of CAP against CYP1A2 and CYP2C9, and Zhang

TABLE 3

Comparison of IC_{50} values for SC-mediated inhibition of P450 functional activity

IC_{50} values are expressed in μM.

CUR	Present study	(Volak et al., 2008)[a]	(Appiah-Opong et al., 2007)[b]	(Bamba et al., 2011)[c]	(Oetari et al., 1996)[d]	(Thapliyal and Maru, 2001)[d]
CYP3A4	11.9 ± 3.5	25.3 ± 1.4	16.3 ± 1.7	14.9 ± 1.4		
CYP2C9	14.6 ± 4.6	13.5 ± 1.4	4.3 ± 0.8	6.0 ± 1.4		
CYP1A2	101.7 ± 10.1	95.4 ± 17.1	40.0 ± 12.7	104.6 ± 22.1	2	9.6
CYP2D6	95.4 ± 12.1	63.6 ± 4.8	50.3 ± 2.0	175.0 ± 47.0		
PIP	Present study	(Volak et al., 2008)[a]				
CYP3A4	2.1 ± 0.5	5.5 ± 0.7 (CYP3A)				
CYP1A2	14.2 ± 4.2	29.8 ± 3.6				
CYP2C9	89.6 ± 9.2	40.7 ± 4.1				
CYP2D6	110.4 ± 3.2	>50				
CAP	Present study	(Babbar et al., 2010)[a]	(Zhang et al., 2012)[a]	(Takanohashi et al., 2010)[a]		
CYP3A4	1.8 ± 0.7	12–38	27.2	21.5		
CYP1A2	2.1 ± 0.2	2.1 ± 0.2	17.2			
CYP2C9	11.9 ± 4.2	2.0 ± 0.2	6.1			
CYP2D6	100.0 ± 5.9	18 ± 5	31.5			

[a] Microsomes isolated from livers from human donors, curcuminoid extracts.
[b] Recombinant P450 enzymes expressed in *E. coli* from donated cDNA plasmids.
[c] BD Gentest enzyme high-throughput inhibitor screening kit.
[d] Microsomes isolated from rodent livers.

et al. (2012) showed CAP to be more active against CYP2C9 than against CYP1A2, CYP3A4, and CYP2D6. Nevertheless, if we were to examine the threshold CAP concentrations that attained equivalency of activity to the manufacturer-recommended inhibitors (Fig. 4), CAP would be classified as having stronger activity against CYP1A2 and CYP2C9, a position more in line with the published data.

The clinical significance of our data depends on the dose and bioavailability of the SC, the relative binding affinity of SC to specific P450 in biologic milieu, and the mechanism of inhibition involved. The concentrations of SC employed in the present study were adequate for pharmacologic interventions. CUR, for example, showed chemoprevention and other biochemical modulations at 5 to 50 μM (Zhang et al., 2012; Yallapu et al., 2014), while CAP and PIP had effective pharmacologic concentrations at 1 to 250 μM (Han et al., 2006; Lin et al., 2013; Zhang et al., 2012), and 10 to 100 μM (Han et al., 2008; Volak et al., 2008; Wang et al., 2013), respectively. However, the extremely low peroral bioavailability of CUR, which yielded low plasma concentrations in the nM range (Sharma et al., 2001), could present a challenge for this SC to be applied for systemic P450 inhibition. Maximum CAP plasma concentrations after consumption of 5 g of chilli peppers or administration of a cutaneous patch (640 μg/cm^2 of CAP) were also relatively low, at 8.1 nM (Chaiyasit et al., 2009) and 58 nM (Babbar et al., 2009), respectively. To our best knowledge, there has been no report on the bioavailability of PIP, but given its structural similarity to CUR and CAP (Suresh and Srinivasan, 2006; Suresh and Srinivasan, 2010), PIP is likely to also present in the nM range after oral administration.

Although significant systemic P450 functional inhibition appears unattainable, given the IC_{50} values are in the μM range, published reports involving the ingestion of relatively high doses of purified SC have indicated otherwise. CUR administered orally at 60 mg/kg/day for 4 days has been shown to affect intestinal P450 protein expression and increase midazolam bioavailability in the rat model (Zhang et al., 2007). PIP at a single oral dose of 50 mg/kg could inhibit hepatic CYP3A4 activity in the mouse model, while a higher dose of 100 mg/kg significantly increased and prolonged the plasma levels of coadministered docetaxel, an anticancer drug (Makhov et al., 2012). Oral administration of CAP at 30 mg/kg to the Wistar rats has also significantly increased the bioavailability and maximum plasma concentration of coadministered phenytoin, an antiepileptic drug of narrow therapeutic index and a substrate of CYP2C9 (Hirotani et al., 2007).

The small intestine is also a major site of P450 metabolic activities, at levels comparable to those in the liver (Ortiz de Montellano, 1995; Wacher et al., 1998). Considering the volume of gastrointestinal (GI) fluid to be approximately 8 L (Lawson, 2003), a typical Indian diet comprising 0.87 g/day of turmeric (3%–5% CUR) (Uma Pradeep et al., 1993) would result in a CUR concentration as high as 10 μM in the GI tract. Likewise, populations that use black pepper regularly in their diets may consume about 0.34 g/day of black pepper (5%–9% PIP) (Bhardwaj et al., 2002), which translates to approximately 7.5 to 13.8 μM of PIP in the GI tract. CAP content in red hot peppers ranges from 0.1% to 1% (Govindarajan and Sathyanarayana, 1991), and a typical Indian or Thai diet involving a daily CAP intake of about 9 mg (Rumsfield and West, 1991) would introduce 3 μM of CAP into the GI fluid. High daily intake of CAP is not unusual. In some Southeast Asian countries, the average daily per capita consumption of CAP is as high as 50 mg (Buck and Burks, 1983) while residents in some parts of the People's Republic of China are reported to consume up to 150 mg of CAP daily (An et al., 1996).

It is thus not inconceivable that orally administered SC could present at sufficiently high levels to produce significant interactions with intestinal P450 enzymes; CYP3A4 and CYP2C9 could be particularly susceptible, with CYP1A2 also potentially sensitive to modulation by CAP and PIP. On this basis, caution may be exercised concerning the coconsumption of SCs and drugs whose metabolism and bioavailability are governed by CYP3A4, CYP1A2, and/or CYP2C9 activities. Undesirable SC–drug interactions could be compounded when the three SCs are consumed together, which is common in many Asian cuisines.

In conclusion, data from the present study demonstrate that PIP and CAP are relatively strong inhibitors of at least three major human P450 enzymes: CYP2C9, CYP1A2, and CYP3A4. CUR is a strong inhibitor of CYP2C9 and CYP3A4. Of the three SCs, CAP has the strongest potential as a P450 inhibitor.

Authorship Contributions

Participated in research design: Shamsi, Lim.
Conducted experiments: Shamsi, Tran, R. Tan, Z. Tan.
Contributed new reagents or analytic tools: Lim.
Performed data analysis: Shamsi, Lim.
Wrote or contributed to the writing of the manuscript: Shamsi, Lim.

References

An D, Yao ZY, and He GY (1996) With fluoride medium–pepper in fluorine pollution status in Guizhou. *Endemic Dis Bull* **11**:55–57.

Appiah-Opong R, Commandeur JNM, van Vugt-Lussenburg B, and Vermeulen NPE (2007) Inhibition of human recombinant cytochrome P450s by curcumin and curcumin decomposition products. *Toxicology* **235**:83–91.

Babbar S, Chanda S, and Bley K (2010) Inhibition and induction of human cytochrome P450 enzymes in vitro by capsaicin. *Xenobiotica* **40**:807–816.

Babbar S, Marier JF, Mouksassi M-S, Beliveau M, Vanhove GF, Chanda S, and Bley K (2009) Pharmacokinetic analysis of capsaicin after topical administration of a high-concentration capsaicin patch to patients with peripheral neuropathic pain. *Ther Drug Monit* **31**:502–510.

Bamba Y, Yun YS, Kunugi A, and Inoue H (2011) Compounds isolated from *Curcuma aromatica Salisb.* inhibit human P450 enzymes. *J Nat Med* **65**:583–587.

Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK, and Fromm MF (2002) Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther* **302**:645–650.

Buck SH and Burks TF (1983) Capsaicin: hot new pharmacological tool. *Trends Pharmacol Sci* **4**: 84–87 DOI: 10.1016/0165-6147(83)90297-3.

Chaiyasit K, Khovidhunkit W, and Wittayalertpanya S (2009) Pharmacokinetic and the effect of capsaicin in *Capsicum frutescens* on decreasing plasma glucose level. *J Med Assoc Thai* **92**: 108–113.

Cheng A-L, Hsu C-H, Lin J-K, Hsu MM, Ho YF, Shen T-S, Ko JY, Lin JT, Lin BR, Ming-Shiang W, et al. (2001) Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* **21** (4B):2895–2900.

Govindarajan VS and Sathyanarayana MN (1991) Capsicum—production, technology, chemistry, and quality. Part V. Impact on physiology, pharmacology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. *Crit Rev Food Sci Nutr* **29**:435–474.

Han EH, Kim HG, Choi JH, Jang YJ, Lee SS, Kwon KI, Kim E, Noh K, Jeong TC, Hwang YP, et al. (2012) Capsaicin induces CYP3A4 expression via pregnane X receptor and CCAAT/enhancer-binding protein β activation. *Mol Nutr Food Res* **56**:797–809.

Han Y, Chin Tan TM, and Lim LY (2008) In vitro and in vivo evaluation of the effects of piperine on P-gp function and expression. *Toxicol Appl Pharmacol* **230**:283–289.

Han Y, Tan TM, and Lim LY (2006) Effects of capsaicin on P-gp function and expression in Caco-2 cells. *Biochem Pharmacol* **71**:1727–1734.

Hemalswarya S and Doble M (2006) Potential synergism of natural products in the treatment of cancer. *Phytother Res* **20**:239–249.

Hirotani Y, Yamamoto K, Tomishi H, Nishihori T, Umeda T, and Tanaka K (2007) Effects of capsaicin on the pharmacokinetics of digoxin, phenytoin and theophylline in rats. *Jpn J Ther Drug* **24**:23–28.

Hismiogullari AA, Hismiogullari SE, Karaca O, Sunay FB, Paksoy S, Can M, Kus I, Seyrek K, and Yavuz O (2015) The protective effect of curcumin administration on carbon tetrachloride (CCl_4)-induced nephrotoxicity in rats. *Pharmacol Rep* **67**:410–416.

Ide H, Tokiwa S, Sakamaki K, Nishio K, Isotani S, Muto S, Hama T, Masuda H, and Horie S (2010) Combined inhibitory effects of soy isoflavones and curcumin on the production of prostate-specific antigen. *Prostate* **70**:1127–1133.

Kato R, Higashitani A, Irie T, Kusukawa Y, Yamamoto Y, Nakagawa M, Urashima Y, Nagata M, Hayashi T, Ijiri Y, et al. (2012) Influence of capsaicin on fluctuation of digoxin pharmacokinetics in lipopolysaccharide-treated rats. *Xenobiotica* **42**:798–807.

Kaur H, Patro I, Tikoo K, and Sandhir R (2015) Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy. *Neurochem Int* **89**:40–50.

Kong WM, Chik Z, Ramachandra M, Subramaniam U, Aziddin RE, and Mohamed Z (2011) Evaluation of the effects of *Mitragyna speciosa* alkaloid extract on cytochrome P450 enzymes using a high throughput assay. *Molecules* **16**:7344–7356.

Lawson DM (2003) A model for visualizing fluid handling by the gastrointestinal tract. *Adv Physiol Educ* **27**:87–88.

Lin CH, Lu WC, Wang CW, Chan YC, and Chen MK (2013) Capsaicin induces cell cycle arrest and apoptosis in human KB cancer cells. *BMC Complement Altern Med* **13**:46.

Makhov P, Golovine K, Canter D, Kutikov A, Simhan J, Corlew MM, Uzzo RG, and Kolenko VM (2012) Co-administration of piperine and docetaxel results in improved anti-tumor efficacy via inhibition of CYP3A4 activity. *Prostate* **72**:661–667.

Nayadu S, Behera D, Sharma M, Kaur G, and Gudi G (2013) Fluorescent probe based CYP inhibition assay: A high throughput tool for early drug discovery screening. *Int Pharm Pharm Sci* **5**:303–307.

Oetari S, Sudibyo M, Commandeur JN, Samhoedi R, and Vermeulen NP (1996) Effects of curcumin on cytochrome P450 and glutathione *S*-transferase activities in rat liver. *Biochem Pharmacol* **51**:39–45.

Ortiz de Montellano PR (1995) Cytochrome P450: Structure, Mechanism, and Biochemistry. Plenum, New York.

Pea F and Furlanut M (2001) Pharmacokinetic aspects of treating infections in the intensive care unit: focus on drug interactions. *Clin Pharmacokinet* **40**:833–868.

Uma Pradeep K, Geervani P, and Eggum BO (1993) Common Indian spices: nutrient composition, consumption and contribution to dietary value. *Plant Foods Hum Nutr* **44**:137–148.

Rodríguez-Antona C, Donato MT, Boobis A, Edwards RJ, Watts PS, Castell JV, and Gómez-Lechón MJ (2002) Cytochrome P450 expression in human hepatocytes and hepatoma cell lines: molecular mechanisms that determine lower expression in cultured cells. *Xenobiotica* **32**:505–520.

Rumsfield JA and West DP (1991) Topical capsaicin in dermatologic and peripheral pain disorders. *DICP* **25**:381–387.

Sharma RA, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, Pirmohamed M, Marnett LJ, Gescher AJ, and Steward WP (2001) Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. *Clin Cancer Res* **7**:1894–1900.

Snawder JE and Lipscomb JC (2000) Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. *Regul Toxicol Pharmacol* **32**:200–209.

Suresh D and Srinivasan K (2006) Influence of curcumin, capsaicin, and piperine on the rat liver drug-metabolizing enzyme system in vivo and in vitro. *Can J Physiol Pharmacol* **84**:1259–1265.

Suresh D and Srinivasan K (2010) Tissue distribution & elimination of capsaicin, piperine & curcumin following oral intake in rats. *Indian J Med Res* **131**:682–691.

Takanohashi T, Isaka M, Ubukata K, Mihara R, and Bernard BK (2010) Studies of the toxicological potential of capsinoids, XIII: inhibitory effects of capsaicin and capsinoids on cytochrome P450 3A4 in human liver microsomes. *Int J Toxicol* **29**(2, Suppl)22S–26S.

Thapliyal R and Maru GB (2001) Inhibition of cytochrome P450 isozymes by curcumins in vitro and in vivo. *Food Chem Toxicol* **39**:541–547.

Trubetskoy O, Marks B, Zielinski T, Yueh MF, and Raucy J (2005a) A simultaneous assessment of CYP3A4 metabolism and induction in the DPX-2 cell line. *AAPS J* **7**:E6–E13.

Trubetskoy OV, Gibson JR, and Marks BD (2005b) Highly miniaturized formats for in vitro drug metabolism assays using vivid fluorescent substrates and recombinant human cytochrome P450 enzymes. *J Biomol Screen* **10**:56–66.

Venkatesh S, Durga KD, Padmavathi Y, Reddy BM, and Mullangi R (2011) Influence of piperine on ibuprofen induced antinociception and its pharmacokinetics. *Arzneimittelforschung* **61**: 506–509.

Volak LP, Ghirmai S, Cashman JR, and Court MH (2008) Curcuminoids inhibit multiple human cytochromes P450, UDP-glucuronosyltransferase, and sulfotransferase enzymes, whereas piperine is a relatively selective CYP3A4 inhibitor. *Drug Metab Dispos* **36**:1594–1605.

Wacher VJ, Silverman JA, Zhang Y, and Benet LZ (1998) Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* **87**: 1322–1330.

Wang YM, Lin W, Chai SC, Wu J, Ong SS, Schuetz EG, and Chen T (2013) Piperine activates human pregnane X receptor to induce the expression of cytochrome P450 3A4 and multidrug resistance protein 1. *Toxicol Appl Pharmacol* **272**:96–107.

Westerink WM and Schoonen WG (2007) Cytochrome P450 enzyme levels in HepG2 cells and cryopreserved primary human hepatocytes and their induction in HepG2 cells. *Toxicol In Vitro* **21**:1581–1591.

Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, and Ball SE (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. *Drug Metab Dispos* **32**:1201–1208.

Yallapu MM, Khan S, Maher DM, Ebeling MC, Sundram V, Chauhan N, Ganju A, Balakrishna S, Gupta BK, Zafar N, et al. (2014) Anti-cancer activity of curcumin loaded nanoparticles in prostate cancer. *Biomaterials* **35**:8635–8648.

Yan YD, Marasini N, Choi YK, Kim JO, Woo JS, Yong CS, and Choi HG (2012) Effect of dose and dosage interval on the oral bioavailability of docetaxel in combination with a curcumin self-emulsifying drug delivery system (SEDDS). *Eur J Drug Metab Pharmacokinet* **37**:217–224.

Zhai XJ, Shi F, Chen F, and Lu YN (2013) Capsaicin pretreatment increased the bioavailability of cyclosporin in rats: involvement of P-glycoprotein and CYP 3A inhibition. *Food Chem Toxicol* **62**:323–328.

Zhang L, Wei MJ, Zhao CY, and Qi HM (2008) Determination of the inhibitory potential of 6 fluoroquinolones on CYP1A2 and CYP2C9 in human liver microsomes. *Acta Pharmacol Sin* **29**:1507–1514.

Zhang QH, Hu JP, Wang BL, and Li Y (2012) Effects of capsaicin and dihydrocapsaicin on human and rat liver microsomal CYP450 enzyme activities in vitro and in vivo. *J Asian Nat Prod Res* **14**:382–395.

Zhang W and Lim LY (2008) Effects of spice constituents on P-glycoprotein-mediated transport and CYP3A4-mediated metabolism in vitro. *Drug Metab Dispos* **36**:1283–1290.

Zhang W, Tan TM, and Lim LY (2007) Impact of curcumin-induced changes in P-glycoprotein and CYP3A expression on the pharmacokinetics of peroral celiprolol and midazolam in rats. *Drug Metab Dispos* **35**:110–115.

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