Title: Curcumin, piperine and capsaicin: a comparative study of spice-mediated inhibition of human cytochrome P450 isozyme activities

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Running title: **Comparative study of spice-mediated inhibition of human CYP**

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Abbreviations: CUR, curcumin; CAP, capsaicin; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; HLM, human liver microsomes; KCZ, ketoconazole; NPV, α-napthoflavone; PIP, piperine; QDN, quinidine; SC, spice components; SPZ, sulfaphenazole;
Abstract

Inhibition of the cytochrome P450 (CYP) enzymes has been shown to enhance the metabolism of drugs that are CYP 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 CYP enzymes, but the selection of which SC to be prioritised for further development as adjuvant will depend on the ranking order of inhibitory potential of the SCs on specific CYP isozymes. This paper aimed to utilise common human recombinant enzyme platforms to provide a comparative evaluation of the inhibitory activities of CUR, PIP and CAP on the principal drug-metabolising CYP 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 3 SCs exhibited weak activity towards 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 3 SCs, CAP therefore has the strongest potential for further development into an inhibitor of multiple CYPs for use in the clinic. Data from this study is also useful for managing potential drug-SC interactions.
Introduction

Co-administration of an agent that inhibits or induces the cytochrome P450 (CYP) Phase I drug-metabolizing enzyme systems is an important cause of adverse events and therapeutic failures for drugs with a narrow therapeutic index (Hemaiswarya and Doble, 2006; Pea and Furlanut, 2001). However, where the CYP-modulating agent is intentionally co-administered 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 CYP-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 CYP-modulating agents, such as the spice components (SCs), curcumin (CUR), piperine (PIP) and capsaicin (CAP) (Figure 1), can enjoy higher acceptance than novel compounds.

CUR, a polyphenolic component of turmeric (Curcuma longa), has promising therapeutic potential as an anti-cancer (Ide et al., 2010), anti-oxidant (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 and Lim, 2008; Zhang et al., 2007). 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 perorally to Sprague Dawley rats at 60 mg/kg once daily over 4 days has resulted in the down-regulation of intestinal CYP3A to levels significant enough to affect the pharmacokinetic parameters of co-administered midazolam (Zhang et al., 2007). The enhancement of docetaxel bioavailability in the rat model by CUR, co-administered 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 CYP enzymes. Unlike CUR, PIP is a non-competitive inhibitor of CYP3A4, and a less potent inhibitor of CYP1A2 and CYP2D6 (Volak et al., 2008). *In vivo*, PIP co-administered perorally 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 CYP isozymes (Han et al., 2012; Kato et al., 2012; Takanohashi et al., 2010). CAP at dietary concentrations may not be a CYP inhibitor, but at concentrations higher than 2.0 µM, it has been shown to inhibit CYP2D6 and CYP1A2 activities sufficiently to impact on drug metabolism (Babbar et al., 2010). CAP may also modify the pharmacokinetic profiles of drugs that are CYP3A substrates (Hirotani et al., 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 CYP inhibitors capable of interacting with drugs. The clinical potentials of PIP (Volak et al., 2008; Wang et al., 2013), CUR (Volak et al., 2008; Zhang et al., 2007) and CAP (Zhai et al., 2013; Zhang et al., 2012) as CYP inhibitors have also been examined. The 3 SCs are interesting because they share similarities in chemical structures (Figure 1) and are complementary in taste, often co-existing in many Asian cuisines. On this basis, we hypothesized that CUR, PIP and CAP
would exhibit comparable inhibitory activities against the major drug-metabolising CYP 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 utilising 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 CYP activity is to be regulated.

The aim of the present study was to address this information gap by comparing the CYP-inhibitory effectiveness of CUR, PIP and CAP using common enzyme platforms, and to ascertain 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 biological 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 prioritised for further development into a pharmaceutical CYP inhibitor.
Materials and Methods

Materials

Analytical graded dimethyl sulfoxide (DMSO) and acetonitrile (ACN) were purchased from Ajax Finechem (Taren Point, NSW, Australia). PIP (≥97%) was from SAFC Global (St Louis, MO, USA). α-naphthoflavone, ketoconazole (≥98%), sulfaphenazole (≥99%), CUR (≥94), CAP (≥95%) and quinidine were purchased from Sigma Chemicals (Castle Hill, NSW, Australia). CYP inhibition assays were conducted using the Vivid CYP1A2, CYP3A4, CYP2C9 and CYP2D6 Blue Screening Kits purchased from Invitrogen (Carlsbad, CA, USA). The kits contained microsomes from baculovirus-infected cells co-expressing 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. Deionised water was used throughout.

Test and control samples

Test samples with SC concentration range of 0 to 120 μM, the upper concentration limited by the SC solubility in the medium, were prepared by dissolving the SC in a pH 8 potassium phosphate buffer (reaction buffer supplied by 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 CYP inhibition (Trubetskoy et al., 2005b).

**CYP Inhibition Assays**

Assays were conducted as triplicate independent experiments for each SC according to the Vivid™ assay protocols provided by the manufacturer (https://tools.thermofisher.com/content/sfs/brochures/VividScreeningKitManual24Apr20121.pdf). All enzyme reactions were conducted under conditions shown to be linear with respect to incubation time, 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 co-incubation 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, and the background fluorescence was measured (λ\text{ex} 405 nm, λ\text{em} 460 nm, FLUOstar OPTIMA, BMG LABTECH, Victoria, Australia) after incubation for 10 min 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 min 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 4 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

$IC_{50}$ value for each SC-CYP pair was determined from non-linear regression analysis of residual enzyme activity versus SC concentration. Data obtained from triplicate independent experiments are expressed as mean ± standard deviation (SD). Enzyme activities at two different inhibitor concentrations (Figure 2) were compared using the paired t-test. Data for each SC-CYP interaction (Figure 3, 4 and 5), and the $IC_{50}$ values of the 3 SCs for each CYP isozyme were analysed using the one-way analysis of variance (ANOVA) with the Tukey’s test applied post-hoc for paired comparison of means. $p \leq 0.05$ signifies significance (SPSS 16.0, SPSS Inc., Chicago, IL, USA).
Results

Integrity of CYP enzyme systems

All 4 recombinant human CYP 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. CYP activity was significantly reduced, but not negated, by co-incubation of the specified substrate and inhibitor at the concentrations recommended by the manufacturer (Figure 2). The extent of CYP 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 4 recombinant enzyme systems was intact, and that they could be used to compare the CYP-modulating activity of the SCs.

CUR-mediated CYP inhibition

CUR was an inhibitor of all 4 recombinant enzymes, its effectiveness was dependent on its concentration and the type of CYP isozyme employed (Figure 3). Of the 4 CYPs, CUR was most effective at inhibiting CYP3A4, almost obliterating the enzyme function at concentrations $\geq 60 \mu M$. It was less effective against CYP1A2, where residual enzyme levels $\geq 40\%$ were observed even at 120 $\mu M$ of CUR (Figure 3). Compared to the established CYP inhibitors, CUR produced comparable inhibitions at a lower concentration than SPZ (15 $\mu M$ vs. 20 $\mu M$) for CYP2C9, and at higher concentrations than KCZ (30 $\mu M$ vs. 5 $\mu M$) and QDN (120 $\mu M$ vs. 0.6 $\mu M$) 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 (Figure 3).
**PIP-mediated CYP inhibition**

PIP also exhibited inhibitory activity against all 4 recombinant enzymes in a manner that was influenced by its concentration and the CYP isoenzyme employed (Figure 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 vs. 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 vs. 20 µM) and NPV (30 µM vs. 1.7 µM) for CYP2C9 and CYP1A2, respectively, and the PIP-mediated inhibitory action on these two CYPs showed levelling 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 CYP inhibition**

CAP was an effective inhibitor of all 4 recombinant isozymes, lowering the enzyme activities to match those seen with the established inhibitors (Figure 5), although CAP achieved this at relatively higher concentrations. The exception was 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 CYP inhibition

IC₅₀ values determined from the non-linear regression analysis of residual enzyme activity versus SC concentration were in the µM range for all 3 SCs (Table 2). Based on the relative IC₅₀ values, the ranking order of inhibitory potential against CYP1A2 was CAP > PIP > CUR, while the 3 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 the CYP2C9, the inhibitory potential being > 6-fold higher than that observed with PIP.

IC₅₀ values for the manufacturer-recommended inhibitors have been published for a variety of enzyme platforms employed (Table 2). When compared against these IC₅₀ values, CUR, PIP and CAP were relatively weaker CYP inhibitors, although CAP has the potential to match the potency of SPZ in inhibiting CYP2C9.
Discussion

The aim of this study was to evaluate the comparative capability of CUR, PIP and CAP to inhibit 4 major human CYP isoymes responsible for drug bioavailability and disposition. Studies investigating SC-mediated inhibition of CYP functional activities are not new. However, as shown in Table 3, the published \(IC_{50}\) data for a SC-CYP 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 CYP-inhibitory potency of the SC.

In this study, we employed a common high throughput CYP enzyme assay kit to evaluate the 3 SCs against a specific CYP activity. The recombinant enzyme system may be more costly and less representative of physiological 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 h at room temperature (Trubetskoy et al., 2005b). Fluorescent high throughput screening methods employ fluorescent P450 substrates that are efficiently metabolized by specific P450 isoymes 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 CYP enzyme. On the basis of such direct comparisons, CAP was shown to be the most promising inhibitor of multiple CYPs, and its effects on drug bioavailability should be further examined in clinical trials. CAP did not only exhibit the lowest mean \(IC_{50}\) values against CYP3A4, CYP1A2 and CYP2C9, but it was also the only SC to exhibit CYP-inhibiting activity equivalent to all 4 positive controls at concentrations \(\leq 90\ \mu M\). Despite the recombinant
enzyme systems being less physiologically relevant, there is general consensus between our data and the published IC50 values on the rank order of CYP inhibition by CUR and PIP, with CUR having greater activity against CYP2C9 and CYP3A4 than against CYP2D6 and CYP1A2 (Appiah-Opong et al., 2007; Bamba et al., 2011; Volak et al., 2008), 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 CYP 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 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 (Figure 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 is dependent on the dose and bioavailability of the SC, the relative binding affinity of SC to specific CYP in biological milieu, and the mechanism of inhibition involved. The concentrations of SC employed in the present study were adequate for pharmacological interventions. CUR, for example, showed chemoprevention and other biochemical modulations at 5 to 50 µM (Yallapu et al., 2014; Zhang et al., 2012), while CAP and PIP had effective pharmacological 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
could present a challenge for this SC to be applied for systemic CYP inhibition. Maximum CAP plasma concentrations following consumption of 5 g of chilli peppers or administration of a cutaneous patch (640 µg/cm² 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 CYP functional inhibition appears unattainable, given the IC₅₀ 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 had been shown to affect intestinal CYP 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 co-administered 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 co-administered phenytoin, an anti-epileptic drug of narrow therapeutic index and a substrate of CYP2C9 (Hirotani et al., 2007).

The small intestine is also a major site of CYP metabolic activities, at levels comparable to those in the liver (Ortiz de Montellano, 1995; Wacher et al., 1998). Considering the volume of GI fluid to be approximately 8 L (Lawson, 2003), a typical Indian diet comprising 0.87 g/day of turmeric (3 – 5% CUR) (Pradeep et al., 1993) would result in a CUR concentration as high as 10 µM in the gastrointestinal (GIT). 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
GIT. 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 South-East 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 China are reported to consume up to 150 mg of CAP daily (An et al., 1996).

It is therefore not inconceivable that perorally administered SC could present at sufficiently high levels to produce significant interactions with intestinal CYPs - 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 co-consumption 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 3 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 3 major human CYP enzymes, namely CYP2C9, CYP1A2 and CYP3A4, while CUR is a strong inhibitor of CYP2C9 and CYP3A4. Of the 3 SCs, CAP has the strongest potential as a CYP inhibitor.
Authorship Contributions

*Participated in research design:* Lee-Yong Lim, Suhailli Shamsi

*Conducted experiments:* Suhailli Shamsi, Huong Tran, Renee Seok Jin Tan, Zee Jian Tan.

*Contributed new reagents or analytic tools:* Lee-Yong Lim

*Performed data analysis:* Suhailli Shamsi, Lee-Yong Lim

*Wrote or contributed to the writing of the manuscript:* Suhailli Shamsi, Lee-Yong Lim
References


Footnotes

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.
Figure Legends

**Figure 1:** Chemical structure of (A) curcumin (CUR); (B) piperine (PIP); and (C) capsaicin (CAP).

**Figure 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 mean ± SD (n=3) of residual enzyme activity expressed as percentage of enzyme activity in the absence of inhibitor. ‘*’ denotes statistically different inhibitory activities between the two concentrations of an inhibitor (p < 0.05).

**Figure 3:** CUR-mediated inhibition of CYP1A2, CYP2C9, CYP2D6 and CYP3A4 as compared to naphthoflavone (NPV, 1.7 µM), sulfaphenazole (SPZ, 20 µM), quinidine (QDN, 0.6 µM), and ketoconazole (KCZ, 5 µM), respectively. Data represent mean ± SD (n=3) of enzyme activity, expressed as percentage of enzyme activity in the absence of inhibitor. ‘*’ denotes statistically different inhibitory activity compared to the respective manufacturer-recommended inhibitor (p < 0.05).

**Figure 4:** PIP-mediated inhibition of CYP1A2, CYP2C9, CYP2D6 and CYP3A4 as compared to naphthoflavone (NPV, 1.7 µM), sulfaphenazole (SPZ, 20 µM), quinidine (QDN, 0.6 µM), and ketoconazole (KCZ, 5 µM), respectively. Data represent mean ± SD (n=3) of enzyme activity, expressed as percentage of enzyme activity in the absence of inhibitor. ‘*’ denotes statistically different inhibitory activity compared to the respective manufacturer-recommended inhibitor (p < 0.05).
Figure 5: CAP-mediated inhibition of CYP1A2, CYP2C9, CYP2D6 and CYP3A4 as compared to naphthoflavone (NPV, 1.7 µM), sulfaphenazole (SPZ, 20 µM), quinidine (QDN, 0.6 µM), and ketoconazole (KCZ, 5 µM), respectively. Data represent mean ± SD (n=3) of enzyme activity, expressed as percentage of enzyme activity in the absence of inhibitor. ‘*’ denotes statistically different inhibitory activity compared to the respective manufacturer-recommended inhibitor (p < 0.05).
Table 1: General reaction conditions for the Vivid™ CYP inhibition assays

<table>
<thead>
<tr>
<th>BACULOSOMES®</th>
<th>Substrate</th>
<th>CYP450 (nM)</th>
<th>Vivid™ Substrate (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>EOMCC</td>
<td>5</td>
<td>3</td>
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<tr>
<td>CYP2C9</td>
<td>BOMCC</td>
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<tr>
<td>CYP2D6</td>
<td>EOMCC</td>
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<td>10</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>BOMCC</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

+ 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.
Table 2: IC<sub>50</sub> values (µM) for CUR, PIP, CAP and the manufacturer-recommended inhibitors against CYP1A2, CYP2C9, CYP2D6 and CYP3A4 isozymes activity. ‘*’ denotes significant difference to CUR and ‘#’ denotes significant difference to PIP (p < 0.05). Data represents mean ± SD (n=3).

<table>
<thead>
<tr>
<th>CYP Isozyme</th>
<th>CUR (µM)</th>
<th>PIP (µM)</th>
<th>CAP (µM)</th>
<th>Published IC&lt;sub&gt;50&lt;/sub&gt; (µM) for inhibitor</th>
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<tbody>
<tr>
<td>CYP1A2</td>
<td>101.73 ± 10.07#</td>
<td>14.19 ± 4.15*</td>
<td>2.14 ± 0.22**</td>
<td>0.26 for NPV (Nayadu et al., 2013) +</td>
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<tr>
<td>CYP2C9</td>
<td>14.58 ± 4.57#</td>
<td>89.62 ± 9.17*</td>
<td>11.95 ± 4.24#</td>
<td>10.00 for SPZ (Zhang et al., 2008) ++</td>
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<tr>
<td>CYP2D6</td>
<td>95.42 ± 12.09</td>
<td>110.40 ± 3.23</td>
<td>99.99 ± 5.88</td>
<td>0.50 for QDN (Kong et al., 2011) +</td>
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<td>CYP3A4</td>
<td>11.93 ± 3.49#</td>
<td>2.12 ± 0.45*</td>
<td>1.84 ± 0.71*</td>
<td>0.54 for KCZ (Trubetskoy et al., 2005a) ++</td>
</tr>
</tbody>
</table>

+ recombinant human isozyme; ++ human liver microsomes; +++ DPX-2 cell line
Table 3: Comparison of IC₅₀ values for SC-mediated inhibition of CYP functional activity.

IC₅₀ values are expressed in µM.

<table>
<thead>
<tr>
<th>CUR</th>
<th>Present study</th>
<th>(Volak et al., 2008)¹</th>
<th>(Appiah-Opong et al., 2007)²</th>
<th>(Bamba et al., 2011)³</th>
<th>(Oetari et al., 1996)⁴</th>
<th>(Thapliyal and Maru, 2001)⁴</th>
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</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>11.9 ± 3.5</td>
<td>25.3 ± 1.4</td>
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<td>14.9 ± 1.4</td>
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<td>CYP2C9</td>
<td>14.6 ± 4.6</td>
<td>13.5 ± 1.4</td>
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<td>CYP1A2</td>
<td>101.7 ± 10.1</td>
<td>95.4 ± 17.1</td>
<td>40.0 ± 12.7</td>
<td>104.6 ± 22.1</td>
<td>2</td>
<td>9.6</td>
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<tr>
<td>CYP2D6</td>
<td>95.4 ± 12.1</td>
<td>63.6 ± 4.8</td>
<td>50.3 ± 2.0</td>
<td>175.0 ± 47.0</td>
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<tr>
<th>PIP</th>
<th>Present study</th>
<th>(Volak et al., 2008)¹</th>
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<td>CYP3A4</td>
<td>2.1 ± 0.5</td>
<td>5.5 ± 0.7</td>
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<td>CYP1A2</td>
<td>14.2 ± 4.2</td>
<td>29.8 ± 3.6</td>
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<tr>
<td>CYP2C9</td>
<td>89.6 ± 9.2</td>
<td>40.7 ± 4.1</td>
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<tr>
<td>CYP2D6</td>
<td>110.4 ± 3.2</td>
<td>&gt;50</td>
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<th>CAP</th>
<th>Present study</th>
<th>(Babbar et al., 2010)¹</th>
<th>(Zhang et al., 2012)¹</th>
<th>(Takanohashi et al., 2010)¹</th>
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<td>CYP3A4</td>
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<td>2.1 ± 0.2</td>
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<td>CYP2C9</td>
<td>11.9 ± 4.2</td>
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<tr>
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<td>100.0 ± 5.9</td>
<td>18 ± 5</td>
<td>31.5</td>
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</tbody>
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¹ Microsomes isolated from livers from human donors, curcuminoid extracts.
² Recombinant CYPs expressed in E coli from donated cDNA plasmids
³ BD Gentest enzyme high throughput inhibitor screening kit
⁴ Microsomes isolated from rodent livers
Figure 1
Figure 2

- CYP1A2 activity (%): NPV 1.7 (20%) < NPV 10 (0%)
- CYP2C9 activity (%): SPZ 20 (60%) > SPZ 30 (40%)
- CYP2D6 activity (%): QDN 0.6 (40%) > QDN 10 (10%)
- CYP3A4 activity (%): KCZ 5 (0%) < KCZ 10 (10%)

* indicates statistical significance.
Figure 3