**In vitro** characterization of 4′-(p-toluenesulfonylamide)-4-hydroxychalcone using human liver microsomes and recombinant cytochrome P450s

Boram Lee*1, Zhexue Wu*1, Taeho Lee1, Xue Fei Tan2, Ki Hun Park2, and Kwang-Hyeyon Liu1

1College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu, Republic of Korea and 2Division of Applied Life Science (BK21 Plus), IALS, Gyeongsang National University, Jinju, Republic of Korea

**Abstract**

1. 4′-(p-Toluenesulfonylamide)-4-hydroxychalcone (TSAHC) is a synthetic sulfonylamino chalcone compound possessing anti-cancer properties. The aim of this study was to elucidate the metabolism of TSAHC in human liver microsomes (HLMs) and to characterize the cytochrome P450 (P450) enzymes that are involved in the metabolism of TSAHC.

2. TSAHC was incubated with HLMs or recombinant P450 isoforms (rP450) in the presence of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)-regenerating system. The metabolites were identified and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). P450 isoforms, responsible for TSAHC metabolite formation, were characterized by chemical inhibition and correlation studies in HLMs and enzyme kinetic studies with a panel of rP450 isoforms.

3. Two hydroxyl metabolites, that is M1 and M2, were produced from the human liver microsomal incubations (k_m and V_max values were 2.46 μM and 85.1 pmol/min/mg protein for M1 and 9.98 μM and 32.1 pmol/min/mg protein for M2, respectively). The specific P450 isoforms responsible for two hydroxy-TSAHC formations were identified using a combination of chemical inhibition, correlation analysis and metabolism by expressed recombinant P450 isoforms. The known P450 enzyme activities and the rate of TSAHC metabolite formation in the 15 HLMs showed that TSAHC metabolism is correlated with CYP2C and CYP3A activity. The P450 isoform-selective inhibition study in HLMs and the incubation study of cDNA-expressed enzymes also showed that two hydroxyl metabolites M1 and M2 biotransformed from TSAHC are mainly mediated by CYP2C and CYP3A, respectively. These findings suggest that CYP2C8, CYP2C9, CYP2C19, CYP3A4 and CYP3A5 isoforms are major enzymes contributing to TSAHC metabolism.

**Keywords**

Cytochrome P450, mass spectrometry, metabolism, microsomes, TSAHC

**History**

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**Introduction**

4′-(p-Toluenesulfonylamide)-4-hydroxychalcone (TSAHC; Figure 1) is a sulfonylamino chalcone that has strong inhibitory effects on tyrosinase and α-glucosidase (Seo et al., 2010). TSAHC blocks cellular migration, invasion and multilayer growth mediated by transmembrane 4 L six family member 5 (TM4SF5), which is overexpressed in hepatocarcinoma subjects (Lee et al., 2009). TSAHC treatment suppressed tumor formation in nude mice xenografted with TM4SF5-expressing cells for 4 weeks (Lee et al., 2011). In addition, TSAHC strongly inhibited CYP2J2 enzyme activity, exhibiting a K_i value of 2.03 μM (Lee et al., 2014a). The CYP2J2 isoform is overexpressed in hepatocarcinoma cells and human liver carcinoma tissues (Jiang et al., 2005; Lee et al., 2014b), and it is responsible for the formation of epoxyeicosatrienoic acids from arachidonic acid (Wu et al., 1996). Several studies have demonstrated that epoxyeicosatrienoic acids promote tumor growth, proliferation, adhesion and migration (Chen et al., 2011; Jiang et al., 2005; Jiang et al., 2009). Therefore, TSAHC might inhibit tumorigenesis via the inhibition of both TM4SF5- and/or CYP2J2-mediated tumorigenic proliferation in hepatocarcinoma. Taken together, these data suggest that TSAHC could be a new candidate anti-cancer drug.

Although much is known about the anti-cancer activity of TSAHC, its metabolism in human liver microsomes (HLMs) and with recombinant P450 isoforms has not been studied. This study was performed with the support of early drug...
discovery to identify the metabolic pathway of TSAHC in HLMs. Mass spectral structural identification of the major metabolites of TSAHC was conducted using human liver microsomes and liquid chromatography-tandem mass spectrometry (LC-MS/MS). In addition, we characterized drug-metabolizing enzymes responsible for TSAHC metabolism using a combination of chemical inhibition, correlation analysis in HLMs and metabolism by expressed recombinant P450 isoforms. Such information may be of considerable clinical importance with regard to interindividual variations in TSAHC metabolism and pharmacokinetics.

Materials and methods

Chemicals and reagents

TSAHC and hydroxy-TSAHC (M2) were synthesized by the Gyeongsang National University (Jinju, Korea) with a purity >99.0% (Figure 1). d-Glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH), /C12-nicotinamide adenine dinucleotide phosphate (/C12-NADP+), clomethiazole, a-naphthoflavone, ketoconazole, montelukast, quinidine, thio-TEPA and tranylcypromine were purchased from Sigma-Aldrich (St. Louis, MO). Solvents were high-performance liquid chromatography-mass spectrometry (LC-MS) grade (Fisher Scientific Co., Pittsburgh, PA), and the other chemicals were of the highest quality available. S-Benzynirvanol, pooled HLMs (coded HLM 150) and 10 different human recombinant cytochrome P450 (rP450) isoforms witch contain cytochrome c reductase were purchased from BD Biosciences (Woburn, MA).

Identification of TSAHC metabolites in human liver microsomes

To identify the phase-I metabolites of TSAHC, 0.25 mg/mL of pooled HLMs (HLM 150, BD Biosciences), 0.1 M potassium phosphate buffer (pH 7.4) and 100 μM TSAHC were preincubated at 37°C for 5 min. Microsomal incubation was initiated by the addition of an NADPH-generating system containing 3.3 mM G6P, 500 units/mL G6PDH, 1.3 mM β-NADP+, and 3.3 mM MgCl₂ to the reaction mixtures (final volume 100 μL), and the reaction mixture was further incubated for 60 min at 37°C with agitation. The control incubations were conducted with heat-denatured HLMs (100°C for 30 min). In all experiments, TSAHC was dissolved in acetonitrile, and the final concentration of acetonitrile did not exceed 1% (1% acetonitrile did not affect significantly the TSAHC metabolism). The reaction was terminated by adding a 50 μL ice-cold acetonitrile and centrifuged at 18 000 × g for 5 min. Aliquots (5 μL) of the supernatant were injected in to LC-MS/MS to identify the TSAHC metabolites.

Preliminary experiments showed that the formation of TSAHC metabolites was linear with respect to both time over 30 min and microsomal protein concentrations up to 1.0 mg/mL. Therefore, a 15-min incubation time and a 0.25 mg/mL microsomal protein concentration were selected for further study. The incubation mixtures containing either 5 μL HLMs (5 mg/mL) or cDNA-expressed P450 isoforms (500 pmol/mL) and various concentration of TSAHC (0–100 μM) were reconstituted in 0.1 M phosphate buffer (pH 7.4) and preincubated for 5 min at 37°C. The final incubation volume was 0.1 mL. The reaction was initiated by the addition of the NADPH-generating system and further incubated for 15 min at 37°C. The reaction was terminated by the addition of 50 μL ice-cold acetonitrile containing 60 nM omeprazole as an internal standard (IS), and the incubation mixtures were centrifuged at 20 000 × g for 5 min. Aliquots of the supernatant (2 μL) were analyzed by LC-MS/MS.

Chemical inhibition studies

The inhibitory effects of known P450 inhibitors on the formation of hydroxy-TSAHC (M1 and M2) were evaluated to determine which P450 isoforms are involved in the metabolic pathway. The P450 isoform-selective inhibitors used were a-naphthoflavone (5 μM) for CYP1A2, tranylcypromine (5 μM) for CYP2A6, thio-TEPA (5 μM) for CYP2B6, montelukast (5 μM) for CYP2C8, sulfaphenazole (10 μM) for CYP2C9, S-benzynirvanol (5 μM) for CYP2C19, quinidine (5 μM) for CYP2D6, clomethiazole (20 μM) for CYP2E1 and ketoconazole (1 μM) for CYP3A. Incubations were
performed with inhibitor, pooled HLMs (HLM 150, 0.25 mg/mL), and TSAHC (3 μM). The P450 isofrom activities in the presence of the inhibitors were compared to that of the inhibitor-free controls.

Correlation experiments
TSAHC (10 μM) was incubated with HLMs from 15 different livers to evaluate the correlation of TSAHC metabolism with the activity of individual P450s. The activities of each P450 isofrom were determined using LC-MS/MS as described previously (Kim et al., 2005; Rodrigues, 1999). Isoform-selective reaction markers were used to determine the activity of each P450: phenacetin O-deethylation was used with 50 μM phenacetin (CYP1A2), coumarin 7-hydroxylation with 5 μM coumarin (CYP2A6), bupropion hydroxylation with 50 μM bupropion (CYP2B6), paclitaxel 6′-hydrolysis with 10 μM paclitaxel (CYP2C8), tolbutamide 4-methylhydroxylation with 100 μM tolbutamide (CYP2C9), S-mephenytoin 4-hydroxylation with 100 μM S-mephenytoin (CYP2C19), dextromethorphan O-deethylmatylation with 5 μM dextromethorphan (CYP2D6), chlorozoxazone 6-hydroxylation with 50 μM chlorozoxazone (CYP2E1) and midazolam 1′-hydroxylation with 5 μM midazolam (CYP3A). The correlation coefficients between the formation rates of hydroxy-TSAHC M1 and M2 from TSAHC and the activity of each P450 isoform were calculated by nonparametric regression analysis (Statistica 7, StatSoft Inc., Tulsa, OK). A p value less than 0.05 was considered statistically significant.

LC-MS/MS analysis of TSAHC and its metabolites
For the identification of TSAHC and its metabolites, a Thermo Vantage triple quadrupole mass spectrometer (ThermoFisher Scientific, San Jose, CA) coupled with a Thermo Accela HPLC system was used. Chromatographic separation was performed on a Luna C18 column (2 mm i.d. × 50 mm, 5 μm, 100 Å, Phenomenex, Torrance, CA) with an isocratic mobile phase consisting of acetonitrile and water (45/55, v/v) containing 0.1% formic acid. The flow rate and total run time were 0.2 mL/min and 6 min, respectively. For metabolite identification, mass spectra were recorded by electrospray ionization in a negative mode. The optimum operating conditions were determined as follows: auxiliary gas, 10 Ar; nitrogen gas flow rate, 8 L/min; sheath gas pressure, 35 Ar; capillary temperature, 350°C; vaporizer temperature, 300°C; and spray voltage, 4000 V.

Quantification was performed by selected reaction monitoring (SRM) of the [M–H]− ion and the related product ion for each metabolite using an IS to establish peak area ratios. Detection of the ions was performed by monitoring the transitions of m/z 392 → 237 for TSAHC (30 eV collision energy), 408 → 237 for hydroxy-TSAHC M1 (30 eV collision energy), 408 → 253 for hydroxy-TSAHC M2 (30 eV collision energy) and 342 → 203 for omeprazole (IS, 17 eV collision energy). Hydroxyl-TSAHC M1, which is the lack of authentic standard, was quantitated using the calibration curve of hydroxyl-TSAHC M2. Analytical data were processed by ThermoFisher Xcalibur software (version 2.1). The lower limit of quantitation for metabolites was 1 nM. The coefficient of variation of interassay precision for the analyte was less than 15%.

Data analysis
In the microsomal incubation studies, the apparent kinetic parameters of TSAHC metabolism (Km and Vmax) were determined by fitting a one-enzyme Michaelis–Menten equation [V = VmaxS/(Km + S)], two enzyme model [V = Vmax1S/(Km1 + S) + Vmax2S/(Km2 + S)], a Hill equation [V = VmaxS^n/(Km+S^n)] or a substrate inhibition model [V = Vmax/(1 + Km/S) + [S]/Kcat)]. The calculated parameters were the Michaelis constant (apparent Km), the maximum rate of formation (Vmax), the intrinsic clearance (CLint, which is equal to Vmax/Km), the substrate inhibition constant (Ksi), and the hill coefficient (n) where [S] is the substrate concentration. Results are expressed as the mean ± S.D. of estimates obtained from pooled HLMs or recombinant P450 isoforms in triplicate experiments. Percentage inhibition was calculated as the ratio of metabolite formation in the presence and absence of the specific inhibitor. Calculations were performed using WinNonlin software (Pharsight, Mountain View, CA).

Results and discussion
After HLMs were incubated with TSAHC in the presence of an NADPH-generating system, two metabolites (M1 and M2) were found by LC-MS/MS (Figures 1 and 2). The retention times for TSAHC, M1 and M2 were approximately 4.2, 1.6 and 2.7 min, respectively. Product ion scan analysis of TSAHC and its two metabolites produced the informative and prominent product ions for structural elucidation. The MS/MS spectrum of TSAHC, having a deprotonated molecular ion [M–H]− at m/z 392, showed major fragment ions at m/z 237 due to the sulfonamide bond cleavage and two minor peaks at m/z 246. The loss of the cinnamaldehyde group was also represented at m/z 155 (tosyl moiety, Figure 2A). Metabolites M1 and M2 were tentatively identified as hydroxy-TSAHC (Figure 2B and C). The mass spectra of M1 and M2 contain a deprotonated molecular ion peak [M–H]− at m/z 408, suggesting one oxygen atom was inserted into the formation (Vmax), the intrinsic clearance (CLint, which is equal to Vmax/Km), the substrate inhibition constant (Ksi), and the hill coefficient (n) where [S] is the substrate concentration. Results are expressed as the mean ± S.D. of estimates obtained from pooled HLMs or recombinant P450 isoforms in triplicate experiments. Percentage inhibition was calculated as the ratio of metabolite formation in the presence and absence of the specific inhibitor. Calculations were performed using WinNonlin software (Pharsight, Mountain View, CA).

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Next, the involvement of the P450-isofrom in the biotransformation of TSAHC was investigated using HLMs and recombinant P450 isoforms. TSAHC was metabolized by HLMs in the presence of an NADPH-generating system, but it was not metabolized in the absence of NADPH, indicating that TSAHC metabolism is P450-dependent (data are not shown). We provide evidence that the formation of hydroxy-TSAHC M1 and M2 from TSAHC is mainly catalyzed by the CYP2C and CYP3A isoform, respectively. First, the formation rate of hydroxy-TSAHC M1 from TSAHC (3 μM) was inhibited by montelukast (90%), sulfaphenazole (40%) and S-benzylvirvanol (37%), selective inhibitors of CYP2C8, 2C9 and 2C19, respectively (Mancy et al., 1996; Suzuki et al., 2002; Walsky et al., 2005). In contrast, the formation rate of hydroxy-TSAHC M1 from TSAHC (3 μM) was inhibited by ketoconazole (50%), a selective CYP3A inhibitor (Figure 3) (Baldwin et al., 1995). The effects of the other P450-isoform-selective inhibitors tested on metabolite formation were less than 20%, confirming that CYP2C and CYP3A play the most prominent role in hydroxy-TSAHC M1 and M2 formation, respectively. Second, TSAHC was incubated at two concentrations (1 and 3 μM) with a panel of cDNA-expressed P450 isoforms. Hydroxy-TSAHC M1 formation was predominantly mediated by CYP2C8, CYP2C9 and CYP2C19, with little contribution from CYP3A4 and CYP3A5 (Figure 4A). Hydroxy-TSAHC M2 was mainly formed by the CYP3A4 isoform, with minor contributions from CYP1A2, CYP2D6 and CYP3A5 isoforms (Figure 4B). Finally, rates of hydroxy-TSAHC M1 and M2 formation were compared to marker activities of each P450 isoform in a series of HLMs containing varying levels of enzyme (Table 1). Paclitaxel 6α-hydroxylation and tolbutamide 4-methylhydroxylation, a marker of CYP2C8 and CYP2C9 activity (Bahadur et al., 2002; Lee et al., 2003), respectively, exhibited high correlation with the rate of hydroxy-TSAHC M1 formation (r = 0.62 and 0.82, respectively) in 15 individual HLMs (Table 1). In contrast, midazolam 1′-hydroxylation activity, a marker of CYP3A activity (Li et al., 2003), exhibited high correlation with the rate of hydroxy-TSAHC M2 formation (r = 0.62; Table 1).

However, the significant correlation we observed between CYP2C9 activity and hydroxy-TSAHC M2 formation in the panel of HLMs tested may not have been caused by the actual involvement of CYP2C9 in hydroxy-TSAHC M2 formation, because our recombinant experiments and chemical inhibition study do not support a significant role of CYP2C9 in hydroxy-TSAHC M2 formation. The observed correlation is probably derived from the significant correlation between the activity of CYP2C9 and CYP3A (Spearman r = 0.71, p < 0.01) in the bank of human livers tested (Supplementary Figure 1). This P450 enzyme interaction activity correlation was also previously reported by Li et al. (2003). They also observed a significant correlation (p < 0.05) between the activities of CYP3A-mediated midazolam 1′-hydroxylation and CYP2C9-mediated diclofenac 4′-hydroxylation in the HLMs bank (Li et al., 2003). These three different approaches collectively demonstrate that CYP2C and CYP3A are major P450s involved in hydroxy-TSAHC M1 and M2 formation, respectively. Based on these findings, a metabolic pathway for TSAHC in human liver microsomes is proposed in Figure 1.
HLMs are shown in Figure 5. The formation of hydroxy-TSAHC M1 from TSAHC was best fitted by the substrate-inhibition model (Figure 5A, left panel). The corresponding Eadie–Hofstee plot had a “hook” in the upper region (Figure 5A, right panel), which is characteristic of substrate inhibition (Liu et al., 2006). The $K_m$ and $K_{si}$ estimated from these data were 2.46 and 30.1 $\mu$M (Table 2), respectively. On the other hand, the rate of hydroxy-TSAHC M2 formation revealed sigmoidal saturation curves that were fitted to a Hill equation (Figure 5B, left panel). The Eadie–Hofstee plot of M2...
formation exhibited a concave relationship, indicating negative cooperativity ($n = 0.89$; Figure 5B, right panel and Table 2). The intrinsic clearance value ($34.6 \mu L/min/mg$ protein) of hydroxy-TSAHC M1 formation was higher than that of hydroxy-TSAHC M2 ($3.21 \mu L/min/mg$ protein; Table 2).

### Table 1. Correlation of the rates of hydroxy-TSAHC M1 and M2 formation from TSAHC (10 µM) with the P450 marker activities in human liver microsomes ($n = 15$).

| Activity                        | P450 isoforms | Hydroxy-TSAHC M1 | Hydroxy-TSAHC M2 |
|--------------------------------|---------------|------------------|------------------|
| Phenacetin O-deethylation      | CYP1A2        | 0.18 ($p = 0.54$) | 0.36 ($p = 0.20$) |
| Coumarin 7-hydroxylation       | CYP2A6        | 0.45 ($p = 0.11$) | 0.02 ($p = 0.94$) |
| Bupropion hydroxylation        | CYP2B6        | 0.59 ($p < 0.05$)* | 0.34 ($p = 0.23$) |
| Paclitaxel 6α-hydroxylation    | CYP2C8        | 0.62 ($p < 0.05$)* | 0.19 ($p = 0.52$) |
| Tolbutamide 4-methylhydroxylation | CYP2C9       | 0.82 ($p < 0.001$)* | 0.69 ($p < 0.01$)* |
| S-Mephenytoin 4-hydroxylation  | CYP2C19       | 0.20 ($p = 0.49$) | 0.18 ($p = 0.53$) |
| Dextromethorphan O-demethylation | CYP2D6      | 0.44 ($p = 0.12$) | 0.47 ($p = 0.09$) |
| Chlorzoxazone 6-hydroxylation  | CYP2E1        | 0.52 ($p = 0.06$) | 0.19 ($p = 0.52$) |
| Midazolam 1′-hydroxylation     | CYP3A         | 0.64 ($p < 0.05$)* | 0.62 ($p < 0.05$)* |

Data were analyzed using Spearman’s nonparametric correlation test. The activity of each P450 isoform was determined using the respective specific substrate probe reaction, as described previously (Kim et al., 2005).

*Statistically significant.

### Table 2. Mean enzyme kinetic parameters of the formation of two hydroxy metabolites (M1 and M2) from TSAHC in human liver microsomes.

| Kinetic parameter | Hydroxy-TSAHC M1 | Hydroxy-TSAHC M2 |
|-------------------|------------------|------------------|
| $V_{max}$         | 85.1             | 32.1             |
| $K_m$             | 2.46             | 9.98             |
| $\text{Cl}_{\text{out}}$ | 34.6           | 3.21             |
| $n$               | 0.89             |                  |
| $K_{si}$          | 30.1             |                  |

$V_{max}$ is expressed as pmol/min/mg protein, $K_m$ and $K_{si}$ are as expressed as µM, $\text{Cl}_{\text{out}}$ is expressed as $V_{max}/K_m$ (µL/min/mg protein), and $n$ is the Hill coefficient.

Figure 5. Kinetics for the formation rate of hydroxy-TSAHC M1 (A) and M2 (B) from TSAHC in human liver microsomes (HLMs). An increasing concentration of TSAHC (0–100 µM) was incubated with HLMs (0.25 mg/mL) and an NADPH-generating system for 15 min at 37 °C. Each point represents the mean of triplicate incubations.

### Declaration of interest

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References

Bahadur N, Leathart JB, Mutch E, et al. (2002). CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. Biochem Pharmacol 64: 1579–89.

Baldwin SJ, Bloomer JC, Smith GJ, et al. (1995). Ketoconazole and sulphaphenazole as the respective selective inhibitors of P4503A and 2C9. Xenobiotica 25:261–70.

Chen C, Wei X, Rao X, et al. (2011). Cytochrome P450 2J2 is highly expressed in hematologic malignant diseases and promotes tumor cell growth. J Pharmacol Exp Ther 336:344–55.

Jiang JG, Chen CL, Card JW, et al. (2005). Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. Cancer Res 65:4707–15.

Jiang JG, Fu XN, Chen CL, et al. (2009). Expression of cytochrome P450 arachidonic acid epoxygenase 2J2 in human tumor tissues and cell lines. Ai Zheng 28:93–6.

Kim MJ, Kim H, Cha II, et al. (2005). High-throughput screening of inhibitory potential of nine cytochrome P450 enzymes in vitro using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 19:2651–8.

Lee B, Kang W, Shon JC, et al. (2014a). Potential of 4’-(p-Toluenesulfonylamine)-4-hydroxychalcone to inhibit the human cytochrome P450 2J2 isozyme. J Korean Soc Appl Biol Chem 57:31–4.

Lee B, Wu Z, Sung SH, et al. (2014b). Potential of decursin to inhibit the human cytochrome P450 2J2 isozyme. Food Chem Toxicol 70:94–9.

Lee CR, Pieper JA, Frye RF, et al. (2003). Tolbutamide, flurbiprofen, and losartan as probes of CYP2C9 activity in humans. J Clin Pharmacol 43:84–91.

Lee SA, Kim YM, Kwak TK, et al. (2009). The extracellular loop 2 of TM4SF5 inhibits integrin alpha2 on hepatocytes under collagen type I environment. Carcinogenesis 30:1872–9.

Lee SA, Lee MS, Ryu HW, et al. (2011). Differential inhibition of transmembrane 4 L six family member 5 (TM4SF5)-mediated tumorigenesis by TSAHC and sorafenib. Cancer Biol Ther 11:330–6.

Li XQ, Bjorkman A, Andersson TB, et al. (2003). Identification of human cytochrome P(450)Is that metabolise anti-parasitic drugs and predictions of in vivo drug hepatic clearance from in vitro data. Eur J Clin Pharmacol 59:429–42.

Liu KH, Kim MG, Lee DJ, et al. (2006). Characterization of ebastine, hydroxyebastine, and carbebastine metabolism by human liver microsomes and expressed cytochrome P450 enzymes: major roles for CYP2J2 and CYP3A. Drug Metab Dispos 34:1793–7.

Mancy A, Dijols S, Poli S, et al. (1996). Interaction of sulphaphenazole derivatives with human liver cytochromes P450 2C: molecular origin of the specific inhibitory effects of sulphaphenazole on CYP 2C9 and consequences for the substrate binding site topology of CYP 2C9. Biochemistry 35:16205–12.

Rodrigues AD. (1999). Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. Biochem Pharmacol 57:465–80.

Seo WD, Ryu YB, Curtis-Long MJ, et al. (2010). Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone. Eur J Med Chem, 45:2010–17.

Suzuki H, Kneller MB, Haining RL, et al. (2002). (+)-N-3-Benzyl-nirvanol and (−)-N-3-benzyl-phenobarbital: new potent and selective in vitro inhibitors of CYP2C19. Drug Metab Dispos 30:235–9.

Walsky RL, Obach RS, Gaman EA, et al. (2005). Selective inhibition of human cytochrome P450C8 by montelukast. Drug Metab Dispos 33: 413–18.

Wu S, Moomaw CR, Tomer KB, et al. (1996). Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. J Biol Chem 271:3460–8.

Supplementary material available online

Supplementary Figure 1.