Short Communication:

The role of bile salt export pump (BSEP) gene repression in drug-induced cholestatic liver toxicity

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Running title: BSEP repression in drug-induced liver toxicity

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No. of words in Results & Discussion: 934

Abbreviations: BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; DILI, drug-induced liver injury; DMSO, dimethyl sulfoxide; FXR, farnesoid x receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LRH-1, liver receptor homolog-1; NRF2, nuclear factor (erythroid 2)-related factor 2.
ABSTRACT

The bile salt export pump (BSEP, ABCB11) is predominantly responsible for the efflux of bile salts and disruption of BSEP function is often associated with altered hepatic homeostasis of bile acids and cholestatic liver injury. Accumulating evidence suggests that many drugs can cause cholestasis through interaction with hepatic transporters. To date, a relatively strong association between drug-induced cholestasis and attenuated BSEP activity has been proposed. However, whether repression of BSEP transcription would contribute to drug-induced cholestasis, is largely unknown. In this study, we have selected 30 drugs, previously reported as BSEP inhibitors, to evaluate their effects on BSEP expression, farnesoid X receptor (FXR) activation, and correlations to clinically reported liver toxicity. Our results indicate that of the 30 BSEP inhibitors, five exhibited potent repression of BSEP expression (≥ 60% repression), ten were moderate repressors (20-60% repression), while others had negligible effects (≤ 20% repression). Of importance, two drugs (troglitazone and benzbromarone), previously withdrawn from the market due to liver injury, are among the potent repressors. Further investigation of the five potent repressors revealed that transcriptional repression of BSEP by lopinavir and troglitazone may occur through their interaction with FXR, while others via FXR-independent yet unidentified pathways. Our data suggests that in addition to functional inhibition, repression of BSEP expression may play an important role in drug-induced cholestatic liver toxicity. Thus, combination of the two would reveal a more accurate prediction of drug-induced cholestasis, than does either repression or inhibition alone.
INTRODUCTION

The primary function of the ATP-binding cassette transporter, bile salt export pump (BSEP, ABCB11), is to facilitate enterohepatic circulation by expelling bile salts from hepatocytes to the bile (Childs et al., 1995). Bile salts are synthesized in the liver via the catabolism of cholesterol; however the majority of bile salts are recycled from the small intestine where they assist in the absorption of dietary fat (Esteller, 2008). BSEP represents one of the rate limiting mechanisms involved in the enterohepatic circulation (Reichen and Paumgartner, 1976). Disruption of BSEP function has been linked to severe forms of cholestasis, characterized by accumulation of bile salts in the liver, jaundice caused by hyperbilirubinemia, and intestinal malabsorption of dietary fat (Ogimura et al., 2011). Cholestasis can occur either through inherited gene mutation or acquired via environmental factor-induced impairment of bile flow (Bull et al., 1998; Maddrey, 2005). The bile salts accumulated in the liver are polar molecules and, at high levels can cause inflammation, apoptosis, and lead to various liver diseases (Stieger, 2009).

Although a close correlation between hereditary defects in BSEP gene and the progressive familial intrahepatic cholestasis type 2 has been firmly established, hereditary forms of cholestasis are clinically rare. In contrast, many xenobiotics including drugs are frequently associated with acquired cholestasis, becoming an increasingly recognized cause of liver disease (Bjornsson and Olsson, 2005). However, the mechanism(s) underlying the involvement of BSEP in the development of drug-induced cholestasis remains unclear. Previous reports have focused primarily on the ability of drugs to inhibit BSEP function, without adequately considering the potential drug-induced perturbation of BSEP expression (Kostrubsky et al., 2003; Morgan et al., 2010). Endpoints for inhibition studies often measure direct efflux competition between bile salts and drugs using plasma-membrane vesicles overexpressing BSEP instead of whole viable cells (van Staden et al., 2012). Some other reports though used rodent or human primary hepatocyte cultures, which provide a physiologically more relevant in
vitro hepatic environment, transporter inhibition was evaluated over a short period of time (10-60 min) after drug exposure (Kostrubsky et al., 2003; Swift et al., 2010). Thus, contribution of BSEP expression in drug-induced cholestasis was largely unexplored in these studies.

Functioning as the major determinant of bile acids secretion and bile formation, BSEP gene is tightly controlled at the transcriptional level by a number of liver enriched transcription factors. The nuclear receptor farnesoid X-receptor (FXR), a ligand-activated nuclear receptor, plays a pivotal role in the inductive expression of BSEP (Ananthanarayanan et al., 2001). Several bile acids, such as chenodeoxycholic acid (CDCA) and lithocholic acid, are endogenous ligands for FXR, and when accumulated in the liver, these bile acids bind to FXR and trigger the expression of the BSEP gene (Makishima et al., 1999). This feedback mechanism ensures the removal of excess bile salts from the hepatocytes. Notably, BSEP expression is partially retained in the liver of FXR-/- mice, suggesting the existence of additional regulators of BSEP expression (Kubitz et al., 2012). Recent evidence reveals that expression of BSEP is also regulated by the nuclear factor erythroid-derived 2-like 2 (NRF2) and the liver receptor homolog-1 (LRH-1). Knockdown of NRF2 or knockout of LRH-1 was associated with decreased expression of BSEP, while activation of NRF2 by oltipraz increased the mRNA expression of BSEP (Song et al., 2008; Weerachayaphorn et al., 2009). Clearly, BSEP expression can be influenced by both endogenous and exogenous chemicals through their interaction with a number of transcription factors. Therefore, it is reasonable to speculate that drug-induced perturbation of BSEP expression in human liver can contribute to the acquired cholestasis.

In this study, we use sandwich-cultured human primary hepatocytes to investigate the role of BSEP repression in drug-induced cholestatic liver injury. Expression of BSEP, FXR, NRF2, and LRH-1 was determined in human hepatocytes upon treatment with an array of clinically used drugs that were reported BSEP inhibitors. Luciferase activation assay for FXR was carried out in HepG2 cells. Correlation of BSEP repression, inhibition, and clinical reported cholestatic injury for these drugs was analyzed. Collectively, our results emphasize that in
addition to inhibition, drug-induced BSEP repression is another critical factor that contributes to drug-induced cholestatic liver injury.

MATERIALS AND METHODS

Chemicals. Troglitazone, erythromycin estolate, cinnarizine, 17-alpha ethynyl estradiol, simvastatin, benzbromarone, and rifampicin were purchased from Sigma-Aldrich (St. Louis, MO). Bosentan was acquired from Waterstone Technology (Carmel, IN). Glyburide and telmisartan were purchased from AK Scientific Inc. (Union City, CA). Pioglitazone, cyclosporine-A, gefitinib, lapatinib, sorafenib, and imatinib were purchased from Selleck Chemicals (Houston, TX). Glimepiride, lopinavir, paclitaxel, pazopanib, indinavir sulfate, fusidic acid sodium salt, and ketoconazole were obtained from Axxora (Farmingdale, NY). Nefazodone, nicardipine hydrochloride, fenofibrate, nelfinavir, saquinavir,itraconazole, ritonavir, and CDCA were generously provided by Dr. James Polli. The Dual-Luciferase reagent was obtained through Promega (Madison, WI). Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Matrigel was received from BD Biosciences (Bedford, MA). All other cell culture reagents were purchased from Invitrogen (Carlsbad, CA) or Sigma-Aldrich (St. Louis, MO).

Plasmids. The FXR expression plasmid and BSEP luciferase reporter vector were kindly provided by Dr. David Mangelsdorf (UT Southwestern Medical Center, Dallas, TX) and Dr. Ruitang Deng (University of Rhode Island School of Pharmacy, Princeton, RI), respectively, as previously described (Lu et al., 2000; Deng et al., 2006). The pRL-TK Renilla luciferase plasmid used to normalize firefly luciferase activities were from Promega.

Human Primary Hepatocytes. Human liver tissues were obtained after surgical resection by qualified pathology staff with donor consent and prior approval from the Institutional Review Board at the University of Maryland, School of Medicine. Hepatocytes were isolated from human liver specimens as described previously (LeCluyse et al., 2005) or obtained from Celsis In Vitro Technologies (Baltimore, MD). Hepatocytes were seeded at $7.5 \times 10^5$ cells/well in 12-well
collagen coated plates and cultured in the sandwich format as described previously (Faucette et al., 2006). Forty-eight hours after seeding, hepatocytes were treated with 0.1% DMSO, or specified compounds for 24 h and 72 h for detection of mRNA and protein expression, respectively. Culture medium was replaced on a daily basis.

**Real-Time PCR.** Total RNA was isolated from hepatocytes using the TRIzol® reagent and reverse transcribed using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) following the manufacturers’ instruction. Primer sequences for real-time PCR are as follows: BSEP: 5’-ACATGCTTGCGAGGACCTTTA-3’ (forward), 5’-GGAGGTTCGTGCACCAGGTA-3’ (reverse); FXR: 5’-ATGGGAATGTTGGCTGAATG-3’ (forward), 5’-CCTGCATGACTTTGTTGTCG-3’ (reverse); and GAPDH: 5’-CCCATCACCATCTTCCAGGAG-3’ (forward), 5’-GTTGTCATGGATGACCTTGGC-3’ (reverse). Target gene mRNA expressions were normalized against that of GAPDH. PCR assays were performed in 96-well optical plates on an Applied Biosystems StepOnePlus Real-Time PCR System (Applied Biosystems) with SYBR Green PCR Master Mix. Fold induction values were calculated according to the equation: fold over control = $2^{\Delta\Delta Ct}$, where $\Delta Ct$ represents the differences in cycle threshold numbers between the target gene and GAPDH, and $\Delta\Delta Ct$ represents the relative change in these differences between control and treatment groups.

**Western Blotting.** Homogenate proteins (40 µg) from hepatocytes were resolved on SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. Subsequently, membranes were incubated with specific antibodies against BSEP (Kamiya, Seattle, WA) or beta-actin (Santa Cruz, CA), followed with HRP-labeled IgG antibodies. Membranes were developed with West Femto chemiluminescent substrate (Thermo, Rockford, IL).

**Transfection in HepG2 cells.** HepG2 cells cultured in 24-well plates at $1 \times 10^5$ cells/well were transfected with the XtremeGene 9 DNA transfection reagent (Roche Applied) 24 h after seeding. The transfection mixes contained the BSEP luciferase reporter plasmid, FXR expression plasmid, and the pRL-TK construct as internal control. Sixteen hours after
transfection, cells were treated with vehicle control (DMSO 0.1%) or CDCA (50 µM) in the
presence and absence of selected compounds for 24 h before harvesting. Cell lysates were
assayed for firefly activities and normalized against the activities of Renilla luciferase using the
Dual-Luciferase Kit (Promega). Data are represented as mean ± S.D. of three individual
transfections.

**MTT assay.** Human primary hepatocytes seeded 7.5 x 10⁴ cells/well in 96-well plates, were
cultured for 48 hours before treatment with BSEP inhibitors at previously indicated
concentrations. A typical MTT assay was carried out as described previously (Wang et al.,
2013). Cell viability was expressed as percent of vehicle control (0.1% DMSO).

**Data Analysis.** Results from real-time PCR and reporter gene assays are expressed as mean ±
standard deviation of triplicate determinations unless otherwise indicated. Statistical analyses
were made where appropriate using one-way ANOVA and chi square tests.

**RESULTS & DISCUSSION**

Preclinical prediction of drug-induced liver injury (DILI), the leading cause of attrition in
drug development, has proven to be challenging. Oftentimes endogenous and/or exogenous
chemicals were accumulated in the hepatocytes and led to inflammation, cholestasis, apoptosis
or necrosis during DILI (Dawson et al., 2012; Yamazaki et al., 2013). Currently, mounting
evidence revealed that inhibition of BSEP function is correlated with a large number of drug-
induced cholestatic liver liabilities (Ogimura et al., 2011). Many BSEP inhibitors have been
identified. Nevertheless, whether transcriptional repression of BSEP expression could contribute
to such correlation remains unclear. Utilizing membrane vesicles from transfected Sf9 insect
cells, Morgan et al., (2010) evaluated more than 200 compounds including FDA approved drugs
and suggested a relatively strong association between the pharmacological interference with
BSEP function and human liver toxicity. In the current study, 30 drugs with a BSEP IC₅₀ value of
25 µM or less from Morgan’s report (Morgan et al., 2010) were analyzed for the BSEP mRNA
expression in human primary hepatocyte sandwich cultures obtained from multiple liver donors.

As shown in Figure 1A, these known BSEP inhibitors could be further classified into three groups based on their effects on BSEP expression in human hepatocytes, which include five drugs as potent repressors of BSEP expression (≥ 60% repression), ten as moderate repressors with decreased BSEP expression at 20-60% of vehicle control, while the rest of the drugs exhibited negligible repression (≤ 20% repression) to moderate induction. Importantly, all drugs were tested at a concentration selected from literature and were not associated with clear cytotoxicity in cultured human primary hepatocytes (Figure 1B), suggesting the selective repression of BSEP was not a non-specific cytotoxic reaction.

Unlike immediate functional inhibition, alteration of BSEP transcription is regulated by a number of transcription factors and can only be reliably measured in physiologically relevant viable cells or in vivo. Among others, FXR has been recognized as one of the key nuclear receptors that mediates drug-induced as well as basal expression of BSEP (Ananthanarayanan et al., 2001). Of the five potent repressors of BSEP, real-time PCR was used to measure the concentration-dependent mRNA expression of BSEP and FXR. As expected, all five drugs exhibited concentration-dependent repression of BSEP mRNA (Figure 2A). Additional western blotting analysis was carried out to examine the expression of BSEP protein upon treatment with each of the five repressors at a single concentration. In agreement with observations at the mRNA level, the identified potent repressors also suppressed BSEP expression at the protein level. On the other hand, expression of FXR was largely unchanged under the same panel of drug treatment (Figure 2B). It is noteworthy to point out that although the expression level of some nuclear receptors correlates well with their target gene expression, prototypical agonists or antagonists often do not affect the expression of their target receptors (Aranda and Pascual, 2001; Li et al., 2012). To further evaluate whether the potent repressors of BSEP can inhibit the activity of FXR, cell-based luciferase assays by co-transfecting FXR expression and BSEP luciferase plasmids were carried out in HepG2 cells. As demonstrated in Figure 2C, at higher
concentrations, both lopinavir and troglitazone significantly reduced the FXR activity that was pre-induced by CDCA, a known endogenous activator of FXR. Intriguingly, a previous report revealed that in Huh7 cells although troglitazone (10 µM) moderately induces the basal expression of BSEP, it dose-dependently represses CDCA-induced BSEP mRNA in a FXR-dependent manner (Kaimal et al., 2009). In contrast, benzbromarone, bosentan or glimepiride only exhibited negligible effects on FXR activity (Fig. 2C). It appears that some of these potent repressors may suppress BSEP expression via FXR-independent mechanisms. Notably, the expression of NRF2 and LRH-1, two transcription factors that have recently been linked with BSEP expression (Song et al., 2008; Weerachayaphorn et al., 2009), was not significantly affected by the same panel of potent repressors in human hepatocytes (Supplemental Figure 1). Further activity assays are warranted for elucidating the role of NRF2 and LRH-1 in drug-induced BSEP repression.

Correlation of the drug-induced BSEP expression profile with clinically reported DILI further reveals that among the five potent repressors, troglitazone and benzbromarone were withdrawn from the market due to severe liver toxicity, and the clinical use of bosentan, an endothelin receptor antagonist, requires monthly monitoring of liver function partly because of its well-known role in intracellular accumulation of cytotoxic bile salts (Fattinger et al., 2001) (Supplemental Table 1). Although it’s difficult to quantify these clinically observed DILI, our findings imply that among the 30 selected BSEP inhibitors, the five potent repressors are generally associated with more severe clinical DILI (Figure 3B) (Chen et al., 2011). Interestingly, the average BSEP IC50 values among the three groups of drugs classified based on BSEP repression were not significantly different (Figure 3C), further supporting the contribution of BSEP expression in DILI. Meanwhile, we do recognize that a number of compounds as known inhibitors of BSEP exhibited moderate induction of BSEP mRNA in the current study. Although it is extremely challenging to dissect the net effects of inhibition vs. induction on the same target molecule, moderate induction of BSEP is unlikely to be a risk factor that enhances BSEP
inhibition-mediated liver injury.

In conclusion, the present study in human primary hepatocytes identifies that a number of known BSEP inhibitors are also potent repressors of this gene. Dual inhibitors and repressors of BSEP are often associated with severe clinically reported DILI. While further systematic and intensive investigations with a larger number of drugs are clearly needed, our current findings indicate that in addition to the well-studied BSEP inhibition, altered expression of this gene should be taken into consideration in predicting drug-induced liver toxicities.

ACKNOWLEDGMENTS

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Garzel, Zhang, Huang, Wang
Conducted experiments: Garzel, Yang, Wang
Performed data analysis: Garzel, Polli, Wang
Wrote or contributed to the writing of the manuscript: Garzel, Yang, Zhang, Huang, Polli, Wang
REFERENCES


FOOTNOTES

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FIGURE LEGENDS

Fig. 1. Repression of BSEP gene expression upon treatment with reported BSEP inhibitors in human primary hepatocytes. BSEP mRNA expression was measured using real-time PCR in hepatocytes from at least three donors upon treatment with BSEP inhibitors as described under Materials and Methods (A). Dotted lines indicate 20% (dark) and 60% (light) BSEP repression compared to vehicle control (0.1% DMSO). A standard MTT assay as described under the Materials and Methods was performed in human primary hepatocytes to monitor cytotoxicities associated with BSEP inhibitors at selected concentrations (B). All the data are expressed as mean ± S.D. (n = 3).

Fig. 2. Concentration-dependent repression of BSEP and FXR. In human primary hepatocytes, five potent repressors of BSEP (≥ 60% BSEP repression, lopinavir, troglitazone, benzbromarone, bosentan, and glimepiride) were investigated in a concentration-dependent manner to determine their effects on BSEP (A) and FXR (B) expression as described under the Materials and Methods. A cell-based luciferase assay was performed in HepG2 cells by co-transfection of the FXR expression and BSEP reporter constructs as detailed under the Materials and Methods (C). The known FXR activator CDCA (50 μM) was used as a positive control. Influence of drugs on FXR activity was determined by comparison between CDCA alone and CDCA + drugs. All the data are expressed as a mean ± S.D. (n=3). ***, p < 0.001; **, p < 0.01; *, p < 0.05.

Fig. 3. Correlation between BSEP repression and clinically reported DILI. (A) Number of DILI drugs in each group of BSEP repressors. (B) The correlation between potent BSEP repression and DILI was analyzed by χ² test. DILI was determined using criteria set forward in Chen et al., (2011). (C) Average BSEP expression and reported IC₅₀ for potent (5), moderate (10), and non-repressors (15). One-way ANOVA with post-hoc test Tukey analysis was performed. ***, p < 0.001.
Figure 1

A

BSEP mRNA Expression in HPH

Fold Change (% DMSO)

DMSO 0.1% Lopinavir 25 μM Troglitazone 100 μM Benzbromarone 15 μM Bosentan 50 μM Fenofibrate 30 μM Glimepiride 10 μM Glibenclamide 50 μM HMG-CoA Reductase 10 μM Itraconazole 6 μM Ketoprofen 15 μM Linoleic acid 15 μM Luminol 10 μM Miconazole 50 μM Modafinil 10 μM Nelfinavir 10 μM Pantoprazole 10 μM Paclitaxel 10 μM Peroxidase 3 nm Phenol Red 10 μM Piroxicam 5 μM Prazosin 5 nm Rasburicase 10 μM Ranitidine 10 μM Ritonavir 10 μM Sodium benzoate 10 μM Tenofovir 10 μM Thioacetamide 10 μM Verapamil 10 μM

B

MTT Assay

Cell Viability (% of DMSO)

DMSO 0.1% Oxidized 40 μM Lopinavir 25 μM Troglitazone 100 μM Benzbromarone 15 μM Bosentan 50 μM Fenofibrate 30 μM Glimepiride 10 μM Glibenclamide 50 μM HMG-CoA Reductase 10 μM Itraconazole 6 μM Ketoprofen 15 μM Linoleic acid 15 μM Luminol 10 μM Miconazole 50 μM Modafinil 10 μM Nelfinavir 10 μM Pantoprazole 10 μM Paclitaxel 10 μM Peroxidase 3 nm Phenol Red 10 μM Piroxicam 5 μM Prazosin 5 nm Rasburicase 10 μM Ranitidine 10 μM Ritonavir 10 μM Sodium benzoate 10 μM Tenofovir 10 μM Thioacetamide 10 μM Verapamil 10 μM
Figure 3

A

Reported Liver Injury

- Represses BSEP Expression ≥ 60%
- Represses BSEP Expression 20-60%
- Represses BSEP Expression ≤ 20%

B

<table>
<thead>
<tr>
<th>BSEP Repression</th>
<th>DILI Drug (+)</th>
<th>DILI Drug (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potent, ≤ 60%; (+)</td>
<td>a=4</td>
<td>b=1</td>
</tr>
<tr>
<td>Moderate or Non-Repressors, ≥ 60%; (-)</td>
<td>c=8</td>
<td>d=17</td>
</tr>
</tbody>
</table>

χ² = 4, p = 0.045
Consistent rate = (a+d)/(a+b+c+d)*100% = 70%

C

<table>
<thead>
<tr>
<th>BSEP Repression</th>
<th>Average Expression (% DMSO)</th>
<th>Average Human BSEP IC₅₀ (μM)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potent, ≥ 60%</td>
<td>30.5 ± 15.5***</td>
<td>15.7 ± 5.9</td>
</tr>
<tr>
<td>Moderate, 20-60%</td>
<td>59.3 ± 23.4***</td>
<td>11.9 ± 6.6</td>
</tr>
<tr>
<td>Non-repression, ≤ 20%</td>
<td>122 ± 45.2</td>
<td>11.6 ± 8.1</td>
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</tbody>
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Supplementary data:
The role of BSEP gene repression in drug-induced cholestatic liver toxicity
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Drug Metabolism & Disposition

Supplemental Table 1
BSEP expression changes as a percentage of DMSO, as depicted in Figure 1 are summarized, along with BSEP IC50 values and reported DILI severity score. Drugs that did not meet the criteria set forth in Chen et al. (2011) and were determined not to have DILI concern are indicated by (--). Abbreviations used for DILI represent withdrawn drugs (W); DILI implicated in the box warning (BW), warnings and precautions (WP), or adverse reactions (AR) sections of label; and most DILI concern (MDC) or less DILI concern (LDC) classifications.

Supplemental Figure 1
Effect of BSEP repressors on NRF2 and LRH-1 mRNA expression. Human primary hepatocytes from two donors were treated with vehicle control (0.1% DMSO) or multiple concentrations of lopinavir, troglitazone, benzbromarone, bosentan, and glimepiride as detailed in “Materials and Methods”. NRF2 (A) and LRH-1 (B) mRNA expression was measured using real-time PCR.
Table 1. Correlation between BSEP expression and DILI

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Average BSEP Expression (% DMSO)</th>
<th>Human BSEP IC&lt;sub&gt;50&lt;/sub&gt; (uM)*</th>
<th>DILI Severity Score**</th>
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<tr>
<td>Represses BSEP ≥ 60%</td>
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<tr>
<td>Lopinavir</td>
<td>21.9 ± 9.5</td>
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<td>8; WP, MDC</td>
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<td>Troglitazone</td>
<td>24.2 ± 8.9</td>
<td>5.9</td>
<td>-2; W, MDC</td>
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<tr>
<td>Benzbromarone</td>
<td>32.4 ± 4.2</td>
<td>17.5</td>
<td>-2; W, MDC</td>
</tr>
<tr>
<td>Bosentan</td>
<td>35.8 ± 5.9</td>
<td>22.0</td>
<td>7; BW, MDC</td>
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<td>Glimepiride</td>
<td>39.2 ± 9.0</td>
<td>15.7</td>
<td>7; AR, LDC</td>
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<td>Represses BSEP 20-60%</td>
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<tr>
<td>Fenofibrate</td>
<td>42.0 ± 7.6</td>
<td>15.3</td>
<td>3; WP, LDC</td>
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<td>Glyburide</td>
<td>45.2 ± 3.5</td>
<td>6.1</td>
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<td>Nelfinavir</td>
<td>47.4 ± 6.5</td>
<td>11.8</td>
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<tr>
<td>Erythromycin estolate</td>
<td>58.8 ± 5.6</td>
<td>13.0</td>
<td>5; WP, MDC</td>
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<td>Gefitinib</td>
<td>59.2 ± 2.2</td>
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<td>Saquinavir</td>
<td>59.6 ± 6.0</td>
<td>4.9</td>
<td>3; WP, LDC</td>
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<td>Ritonavir</td>
<td>60.8 ± 8.5</td>
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<td>Rifampicin</td>
<td>68.1 ± 10.4</td>
<td>25.3</td>
<td>8; WP, MDC</td>
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<td>Cinnarizine</td>
<td>72.8 ± 12.1</td>
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<td>17-alpha ethinyl estradiol</td>
<td>79.1 ± 7.0</td>
<td>14.0</td>
<td>2; AR, LDC</td>
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<tr>
<td>Represses BSEP ≤ 20%</td>
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<tr>
<td>Itraconazole</td>
<td>81.9 ± 20.9</td>
<td>18.0</td>
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<td>Imatinib</td>
<td>86.2 ± 14.3</td>
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<td>Simvastatin</td>
<td>89.4 ± 8.5</td>
<td>24.7</td>
<td>3; WP, LDC</td>
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<tr>
<td>Paclitaxel</td>
<td>95.9 ± 3.5</td>
<td>15.0</td>
<td>--</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>100.9 ± 15.1</td>
<td>16.2</td>
<td>--</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>106.3 ± 13.0</td>
<td>10.3</td>
<td>8; BW, MDC</td>
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<td>Lapatinib</td>
<td>106.3 ± 4.0</td>
<td>6.5</td>
<td>8; BW, MDC</td>
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<td>Indinivir</td>
<td>107.4 ± 14.7</td>
<td>21.2</td>
<td>8; WP, MDC</td>
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<td>Ketoconzole</td>
<td>114.6 ± 25.1</td>
<td>3.4</td>
<td>8; BW, MDC</td>
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<td>Nefazodone</td>
<td>118.2 ± 19.4</td>
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<tr>
<td>Sorafenib</td>
<td>134.4 ± 6.8</td>
<td>8.0</td>
<td>--</td>
</tr>
<tr>
<td>Fusidic Acid</td>
<td>135.5 ± 6.7</td>
<td>10.1</td>
<td>--</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>141.9 ± 13.4</td>
<td>0.4</td>
<td>3; WP, LDC</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>159.4 ± 8.4</td>
<td>7.9</td>
<td>--</td>
</tr>
<tr>
<td>Cyclosporine-A</td>
<td>186.8 ± 19.4</td>
<td>0.9</td>
<td>2; WP, LDC</td>
</tr>
</tbody>
</table>

*BSEP IC<sub>50</sub> values reported by Morgan et al. (2010)

**DILI severity score determined by Chen et al. (2011) and the associated LTKB benchmark dataset (FDA)
Figure 1

A) NRF2 mRNA Expression

B) LRH-1 mRNA Expression