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# Glucuronidation: Driving Factors and Their Impact on Glucuronide Disposition

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## Abstract

Glucuronidation is a well-recognized phase II metabolic pathway for a variety of chemicals including drugs and endogenous substances. Although it is usually the secondary metabolic pathway for a compound preceded by phase I hydroxylation, glucuronidation alone could serve as the dominant metabolic pathway compounds, including some with high aqueous solubility. Glucuronidation involves the metabolism of parent compound by UDP-glucuronosyltransferases (UGTs) into hydrophilic and negatively charged glucuronides that cannot exit the cell without the aid of efflux transporters. Therefore, elimination of parent compound via glucuronidation in a metabolic active cell is controlled by two driving forces; the formation of glucuronides by UGT enzymes and the (polarized) excretion of these glucuronides by efflux transporters located on the cell surfaces in various drug disposition organs.

Contrary to the common assumption that the glucuronides reaching the systemic circulation were destined for urinary excretion, recent evidences suggest that hepatocytes are capable of highly efficient biliary clearance of the gut-generated glucuronides. Furthermore, the biliary- and enteric-

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eliminated glucuronides participate into recycling schemes involving intestinal microbes, which often prolong their local and systemic exposure, albeit at low systemic concentrations. Taken together, these recent research advances indicate that though UGT determines the rate and extent of glucuronide generation, the efflux and uptake transporters determine the distribution of these glucuronides into blood and then to various organs for elimination. Recycling schemes impact the apparent plasma half-life of parent compounds and their glucuronides that reach intestinal lumen, in addition to prolonging their gut and colon exposure.

### Keywords

Glucuronidation; glucuronides; UGT; disposition; recycling; interplay; efflux transporters; uptake transporters

## 1. Introduction

### Glucuronidation Process.

Glucuronidation is an enzyme reaction process catalyzed by UDP-glucuronosyltransferases (i.e., UGTs) in different animals including humans, as well as plants and bacteria (Mackenzie et al., 2003) (Nagar and Blanchard, 2006; Nagar and Remmel, 2006). Glucuronidation process attaches a glucuronide moiety to a substrate making a product that is highly hydrophilic (Radominska-Pandya et al., 1998; Tukey and Strassburg, 2000; Mackenzie et al., 2003; Nagar and Blanchard, 2006; Nagar and Remmel, 2006; Perera et al., 2008). The glucuronides are then often eliminated via bile or urine. Therefore, glucuronidation is considered to be a detoxification process or a defense mechanism that helps humans remove unwanted substances including endogenous substances (e.g., bilirubin), drugs (e.g., SN-38) and other xenobiotics (e.g., environmental toxins) from the body. For example, genetic deficiency related to UGT1A1 could result in hyperbilirubinemia, a disease called Gilbert's syndrome (Radominska-Pandya et al., 1998; Tukey and Strassburg, 2000; Mackenzie et al., 2003; Nagar and Blanchard, 2006; Nagar and Remmel, 2006; Perera et al., 2008). Hence, glucuronidation is an essential biological process in humans, protecting us from excessive accumulation of toxic substances in the body.

Study of the glucuronidation processes started about the same time as the study of cytochrome P450 (or CYP). Initial report of human cytochrome P450 was in 1960s (Reynolds, 1966) and the first human CYP isoform was cloned in 1985 (actually a partial cloning of human CYP2A6) (Phillips et al., 1985). Similarly, human UGT catalyzed reaction was also first reported in the 1960s (Pogell and Leloir, 1961) and the first human UGT was cloned in 1988 (Harding et al., 1988). However, there are major differences between these two enzyme superfamilies (UGTs and CYPs) with respect to the volume of research. A PubMed search, conducted on July 16 of 2016, using the keyword combination of "human cytochrome P450" generated 46,604 hits, whereas the same search using the keyword combination of "human glucuronide" only generated 7257 hits (a keyword combination of "human glucuronidation" only generated 2984 hits). Therefore, we often and quite accurately believe that we collectively know more about CYPs than UGTs.

## Glucuronidation of Drugs and Endogenous Substances.

Despite apparent limitations relative to CYP studies, significant amount of information exists on glucuronidation, especially glucuronidation of drugs by UGTs. Specifically, these enzymes are broadly but unevenly distributed throughout various cells, tissues and organs with heavy concentrations in the first-pass metabolism organs (i.e., liver and intestine) as well as the major elimination organ (i.e., kidney). Glucuronidation serves as the primary elimination pathway for a variety of drugs on the market (Table 1). However, in contrast to a relatively small number of drugs with glucuronidation as primary elimination pathway, for a vast majority of drugs, glucuronidation often occurs as a secondary step after the primary metabolites are produced by phase I reaction such as hydrolysis, hydroxylation, dealkylation, etc. As shown in Table 2, the range of chemical structure that undergoes glucuronidation as secondary step is quite diverse.

Majority of the glucuronides are pharmacologically inactive, however, in certain incidences glucuronides have been shown to be equally or more effective than the parent drug. For example, morphine-6-glucuronide is reported to be 45–61 folds more potent (Frances et al., 1990; Stone et al., 2003) and ezetimibe-glucuronide is reported to be 2–11 folds more potent (Ghosal et al., 2004; Kosoglou et al., 2005; Oswald et al., 2007) than their respective parent compounds. Additionally, some glucuronides can be toxic. For example, many acyl glucuronides have been shown to have high potential toxicities *in vitro* and *in vivo* (Shipkova et al., 2003).

Although not frequently reported, hydrophilic molecules are sometimes also glucuronidated. These molecules are often conjugated with a highly hydrophilic group (e.g., sugar or sulfate) but that did not appear to prevent them from getting glucuronidated. Several flavonoid glycosides are conjugated into glucuronides as reported in Table 3. Furthermore, it is difficult to separate the highly hydrophilic glycosides from their glucuronides in the reverse-phase chromatographic column, which is frequently used to separate drug from its glucuronide(s) during sample analysis. Therefore, mass spectrophotometry is usually employed to analyze glycoside and their glucuronides in the *in vitro* and *in vivo* experimental studies. This need for LC-MS/MS might have been the reason why hydrophilic UGT substrates are more difficult to identify, especially when the hydrophilic moiety is a sugar that is often deconjugated in the ion source of mass spectrometer.

When compared to CYP-catalyzed reactions, an important distinction of glucuronidation is that the metabolites produced are highly hydrophilic molecules that cannot penetrate the cell membrane via passive permeation. Rather, they need the action of various efflux transporters to pump them out of the cells (Jeong et al., 2005b). Hence, the driving force for glucuronidation is different from that of CYP catalyzed reaction. Specifically, it is driven by the twin forces of UGT enzyme present in the cellular endothelial reticulum and efflux transporters present on the cell surface. The efflux transporters can act as a driving force for glucuronidation by controlling the rate of efflux of glucuronides from the cells, which in turn can affect the formation rate. The faster efflux rate can cause the rate of glucuronide formation to increase due to the removal of potentially inhibitory glucuronides. Whereas, the slower efflux rate can cause lower glucuronidation output when compared to the actual glucuronidation capacity based on sub-cellular fractions, possibly using a product-inhibition

feedback mechanism. In vast majority of the cases, these reactions occur in a polarized cell, making the glucuronide excretion polarized and dependent on the distribution of efflux transporter on two polar surfaces of a differentiated cell (Fig 1). For most orally administered drugs, these twin forces are in full effect in intestinal and liver cells, and together they determine the dispositional fate of a glucuronide.

Another distinction of the glucuronidation process is that the corresponding metabolites produced can be reconverted back to the original compound (or aglycone). This process could occasionally occur in mammalian tissues (usually at a very slow rate), but reconversion is extraordinary rapid in the colon when they are in contact with intestinal microflora, which produces a large quantity and variety of glucuronidases that can readily convert glucuronides into aglycones. The aglycones can then be re-absorbed to complete the process of recycling or recirculation. Hence, for glucuronides that are excreted back to the intestinal lumen, they often become bioavailable again following reconversion to the original compound.

There are considerable differences between UGTs and CYPs enzyme systems in terms of the similarity among their respective isoforms and the clinical significance of their genetic polymorphism. UGT enzymes are quite different from CYP in that each UGT subfamily tends to be clustered closely to each other, sometimes sharing the same gene. For example, the human UGT1A family shares the same gene (Guillemette, 2003; Kiang et al., 2005; Mackenzie et al., 2005; Bosch, 2008; Nies et al., 2008; Ginsberg et al., 2010), and utilizes alternative splicing to produce 9 active isoforms (UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10). In contrast to CYPs, clinical significance of polymorphism in UGT isoforms remains mostly unclear; perhaps due to the fact the glucuronide production is dependent on both formation and efflux of glucuronide.

Therefore, the purpose of the present review is to outline how these twin forces of glucuronidation affect the production and biodistribution of glucuronides in various body compartments including intestine, liver, blood, and urine. Discussions related to recently-identified efficient hepatocytes-mediated uptake of glucuronides and various recycling schemes are included because they have impact on the disposition of glucuronides *in vivo*.

## 2. Nomenclature, Mechanisms of Glucuronidation and Organ-specific Distribution of UGTs

### Human UGT Nomenclature.

Based on the amino acid sequence identity, four different families of UGTs are observed in human, namely UGT1, UGT2, UGT3, and UGT8 (Mackenzie et al., 2005). Among these UGT isoforms, UGT1 (UGT1A) and UGT2 (UGT2A and UGT2B) subfamilies are to be considered of paramount importance in terms of imparting drug conjugation ability. Currently, a total of 19 human UGT isoforms are known from both subfamily UGT1 and UGT2. Experimental studies reported that human UGT1A contains 13 distinct individual promoters in chromosome 2q37, which spans approximately 200 kb; whereas human

UGT1B contains six individual promoters on the chromosome 4q13 (Mackenzie et al., 2005).

## Mechanisms of Glucuronidation.

Glucuronidation is one of the most important phase II conjugative reactions, which eliminates predominantly drugs, dietary substances, toxins and endogenous substances. This particular reaction involves the transfer of the glucuronic group from uridine 5'-diphospho-glucuronic acid (UDPGA) to different substrate molecules containing oxygen, nitrogen, sulfur or carboxyl functional groups to generate relatively polar/hydrophilic glucuronide conjugate. In 2010, it was discovered that human UGTs mediate glucuronidation reaction by using a serine hydrolase-like mechanism, which involves two key amino acids histidine and aspartic acid (so-called "catalytic dyad" or "acid base pair") (Radominska-Pandya et al., 2010). Investigations later showed that the glucuronidation reaction involves the formation of a ternary complex of enzyme, substrate and the co-factor UDPGA prior to the formation of ultimate conjugate (Luukkanen et al., 2005).

In addition, based on the inhibition studies using expressed recombinant human UGT isoforms, a compulsory ordered bi bi (i.e., two substrates and two products) kinetic mechanism was proposed (Luukkanen et al., 2005) where the co-factor UDPGA first binds with particular UGT enzyme and then forms a complex with substrate. On the other hand, alternative mechanisms such as random ordered bi bi mechanism were also reported, where binding of the substrate to the enzyme does not require prior binding to UDPGA (Yin et al., 1994). Based on the analysis of Luukkanen et al., these conflicting results were largely observed owing to the presence of multiple UGT enzymes and/or inactivated UGT enzyme in the latter study (Luukkanen et al., 2005).

## Tissue Distribution of UGTs.

Generally UGT is present in humans, other animals (except cat), plants, and bacteria (Court and Greenblatt, 2000). In case of human, it is primarily distributed in different metabolic organs i.e., liver, kidney and intestine etc. (Uchaipichat et al., 2006). Studies showed that approximately 15%, 20% and 35% of marketed drugs are metabolized by three important human UGT isoforms, namely, UGT1A1, 1A4 and 2B7, respectively (Williams et al., 2004). Different human UGT isoforms such as UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15, and 2B17 etc. have been reported to be present in liver (Tukey and Strassburg, 2000; Izukawa et al., 2009). After comparing the mRNA expressions of different UGT isoforms it has been found that, compared to UGT1A isoforms, UGT2Bs are more abundant in the human liver (Ohno and Nakajin, 2009). Among UGT2B subfamily, UGT2B4 and UGT2B15 are the highest expressed UGT isoforms present in liver. In case of UGT1A subfamily, UGT1A1 and UGT1A9 are the most abundant isoform present in liver. Apart from liver, UGT isoforms such as UGT1A8, UGT1A10 and UGT2B17 are predominantly expressed in human colon and intestine. The same study indicated that UGT1A7 is only present in the proximal tissues of the gastrointestinal tract (mainly the esophagus and stomach) (Ohno and Nakajin, 2009).

Since protein expression is considered to more accurately reflect the activity of enzymes, the absolute protein expression levels of some UGT isoforms in human liver were determined using LC-MS/MS in a recent study. According to their report, among all measured isoforms, UGT1A6 had the highest expression level in human liver microsomes. It was also found that there was a low correlation between protein and mRNA quantities for most of the UGT isoforms analyzed except UGT1A6 (high correlation with $r^2$ of more than 0.6 (Ohtsuki et al., 2012). The absolute quantification of different UGT isoforms in rat tissue has not been reported yet.

Many of the studies have been performed to determine the mRNA levels of UGTs in different species such as rat and mouse (Shelby et al., 2003; Mackenzie et al., 2005; Owens et al., 2005; Buckley and Klaassen, 2007). In case of rats, both Ugt1a and Ugt2b subfamilies have been observed to be present. In contrast to human, Ugt1a subfamily containing 10 different isoforms (Ugt1a1, 1a2, 1a3, 1a4, 1a5, 1a6, 1a7, 1a8, 1a9, and 1a10), while Ugt2b subfamily consists of six members, namely, Ugt2b1, 2b2, 2b3, 2b6, 2b8, and 2b12 (Mackenzie et al., 2005; Owens et al., 2005). After comparing the mRNA expression of rat Ugt1 isoforms in different tissues, it was found out that rat Ugt1a isoforms are more prevalent in both liver and intestine compared to other tissues (Shelby et al., 2003). In liver, different isoforms of Ugt1a and Ugt2b subfamilies such as Ugt1a1, 1a3, 1a5, 1a8, 2b1, 2b2, 2b3, 2b6, and 2b12 were present, whereas Ugt1a1, 1a2, 1a3, 1a6 and 1a7 were present in rat intestine. The same study also concluded that only a few Ugt2b subfamily members (Ugt2b3, 2b8 and 2b12) were found in rat intestine (Shelby et al., 2003).

There are similarities and differences in expression of UGTs between rat and mouse. In case of mice, studies indicated that the Ugt1a subfamily contains 14 first exons (Buckley and Klaassen, 2007). Among them only nine enzymes were coded (Ugt1a1, 2, 5, 6a, 6b, 7c, 8, 9, and 10); whereas five were pseudogenes (Ugt1a3, 4, 7a, 7b, and 11). In case of Ugt2b subfamily, only seven Ugt2b genes (Ugt2b1, 2b5, 2b34, 2b35, 2b36, 2b37, and 2b38) were observed to be present in mice. Like rat, mouse liver was also shown to express different Ugt1a isoforms such as Ugt1a1, 1a5, 1a6, 1a9 as well as all Ugt 2b members. In addition, different Ugt subfamily such as Ugt1a6, 1a7c, 2a3, 2b34, and 2b35 are present in mouse gastrointestinal tract (Buckley and Klaassen, 2007).

## 3. Efflux Transporters Involved in Glucuronide Excretion

Phase II metabolites of drugs such as glucuronides, once formed, uses efflux transporters to exit the cell. Since the glucuronides are the substrate of efflux transporters, the two kinetic processes (glucuronidation by UGT and the excretion of glucuronides by efflux transporters) interplay with each other. These interplays are necessary, in large part, due to highly hydrophilic and charged properties of the metabolites, which requires the action of the efflux transporters (e.g., MRPs, BCRP) to exit cells.

### MRPs.

The MRPs are the major efflux transporter family for phase II metabolites, and these transporters are expressed in many epithelial cells (Bera et al., 2002; Meyer zu Schwabedissen and Kroemer, 2011). MRPs share approximately 15% sequence similarities

to that of P-gp (Mdr1), and these efflux transporters are predicted to form a large central hydrophobic core in their active binding region. The core region has two nucleotide-binding domains (NBD1 and NBD2); two membrane-spanning domains (MSD1 and MSD2); six trans-membrane spanning helices; and a linker segment called L1. Some of the MRP subfamily members also contain a membrane-spanning domain zero (MSD0), trans-membrane helices, and a linker zero (L0). The MSD0 and L0 are additional extensions that dangle at the N-terminus that extend extracellularly. Nine of the thirteen members of the multidrug resistant MRP/ABCC family are capable of effluxing both endogenous and exogenous organic anion compounds. Some MRPs, such as MRP1, are additionally capable of transporting neutral organic compounds in the presence of free glutathione (Kruh and Belisnsly, 2003). Collectively, the MRP family of efflux transporter is first known to convene resistance to anticancer agents (Kock and Brouwer, 2012). More recently, the Mrp family of efflux transporters has become known to efflux sulfate, glucuronide, and glutathione metabolites into the interstitial space, bile duct, intestinal lumen and basolateral surface of hepatocytes and enterocytes (Keppler and Konig, 2000; Jemnitz et al., 2010; Keppler, 2011; Kock and Brouwer, 2012).

Among the MRPs, Mrp1, Mrp3, Mrp5, and Mrp6 are densely expressed at the basolateral membrane of an epithelial cell, whereas Mrp2 is found in the apical side of the cell. Mrp4 expression and location depends on the tissue and species (Klaassen and Aleksunes, 2010) (van der Deen et al., 2005). For example, Mrp4 is found in the basolateral membrane of human prostatic glandular cell, but has also been localized to the apical membrane of rat kidney tubule cells. For Mrp7, Mrp8, and Mrp9, their specific locations of expression are less clear and appear to be random (Cai and Gros, 2003; Marquez et al., 2009).

Mrp1 was the first of MRPs to be discovered. It is ubiquitous in epithelial vesicular tissue such as lung and blood-tissue barriers. Mrp1 effluxes glutathione and glucuronide conjugates into the tissue underlying the membrane instead of effluxing these metabolites into the interstitial space. Mrp1 is highly expressed in the intestine and certain other organs such as liver (Cherrington et al., 2002), however, the expression level of Mrp1 varies between rats.

Mrp2 is probably the most widely studied MRP transporter, primarily because at least two MRP2-deficient rat models (Eisai hyperbilirubinuria rats and TR- rats) are available to study the role of MRP2 in drug disposition (Chen and Tiwari, 2011). MRP2 is believed to be responsible for transporting bile acid conjugates. Hepatic deficiency of Mrp2 in rats is linked to low bile acid conjugate excretion. There are various reports of a linkage between disease severity and expression level in both humans and rats. MRP2 is shown to be deficient in the hereditary condition known as Dubin-Johnson syndrome, which causes chronic conjugated hyperbilirubinemia and hepatocytes are not able to excrete conjugated organic anions into bile (Keppler and Konig, 1997). MRP2 in the intestine serves as the first barrier against all toxins entering into the systemic circulation (Jansen et al., 1985; Kamisako et al., 1999; Kamisako et al., 2000; Zamek-Gliszczynski et al., 2011). Mrp2 effluxes organic anions that are conjugated to glutathione, glucuronic acid, or sulfate into the intestinal lumen, thereby eliminating/reducing toxicity exposures to epithelial cells at the tip of the villus part of the

jejunum (Cherrington et al., 2002; Wittgen et al., 2012) (Meyer zu Schwabedissen and Kroemer, 2011).

Mrp3 is very important for basolateral clearance of drugs and other substances in organs such as liver, gut, adrenals, pancreas, and kidney (Zelcer et al., 2001; Kock and Brouwer, 2012). It facilitates the excretion of organic anions, especially bile acids. Importantly, Mrp3 aids in the excretion of glucuronide metabolites into sinusoidal blood within the liver and into portal vein from enterocytes. Studies have shown that while expression level of Mrp3 was unrelated to the excretion of sulfate conjugates, it was proportional to the basolateral clearance of glucuronide conjugates in rats (Borst and Elferink, 2002).

Fewer studies have been conducted on other MRPs. Mrp4 appears to have high affinity for sulfate conjugates of bile acids and steroids (Kock and Brouwer, 2012). Glucuronide and glutathione metabolites also interact with Mrp4 in the liver; however, they have lower affinity in the presence of sulfate conjugates (Russel et al., 2008). Mrp4 is expressed in the prostate, lung, muscle, and pancreas (Klaassen and Aleksunes, 2010). Mrp5 expression is relatively low in a healthy liver. However, in hepatic cholestasis condition, Mrp5 mRNA is up regulated (Kock and Brouwer, 2012). Mrp5 selectively binds to glutathione conjugates and cyclic nucleotides, which are also substrate for Mrp4. Mrp5 is also the main anionic conjugate efflux transporter at the basolateral side and is ubiquitous in many organs (Klaassen and Aleksunes, 2010; Chen and Tiwari, 2011; Kock and Brouwer, 2012).

The physiologic function and the potential involvement in drug resistance of the other Mrps are still under investigation. Mrp6 localizes in the kidney and the basolateral surface of hepatocytes and is mainly involved in transporting glutathione conjugates and substrates such as BQ123, a cyclic-pentapeptide endothelin receptor antagonist (Kool et al., 1999; Madon et al., 2000; Belinsky et al., 2002). Specific transporting mechanisms of Mrp5 and Mrp6 are unclear. Mrp7 is a potassium channel regulator and therefore can be found in various organs, such as pancreas, testis, colon, spinal cord, tonsils, lung, trachea, and skin (Klaassen and Aleksunes, 2010). Mrp7 is capable of effluxing various amphipathic anions such as 17-beta-estradiol-(17-beta-d-glururonide) (Chen et al., 2003b). Mrp8 is ubiquitous throughout many organs and is responsible for nervous responses (Bortfeld et al., 2006). Mrp8 is known to efflux dehydropiandrosteron-3-sulfate, an endogenous precursor of many sex hormones (Chen et al., 2005b; Bortfeld et al., 2006). Mrp9 sequence is about 45–55% similar to that of Mrp5 sequence (Meyer zu Schwabedissen and Kroemer, 2011), but, its specific substrate and organ expression remain unclear so far (Bera et al., 2002).

### BCRP.

The breast cancer resistance protein (Bcrp, ABCG2) was also identified as a hepatic canaliculus drug efflux transport protein, although its role in cancer multidrug resistance has been known for decades (Nakanishi and Ross, 2012). BCRP is densely expressed in the liver, kidney, blood-brain, intestine, and placental barriers (Doyle and Ross, 2003). BCRP's role in biliary excretion of phase II conjugates is just beginning to be understood, partly because it covers a wide range of substrates. Common to the P-gp and MRP family, a single Transmembrane domain (TMD) binds to the terminus of the Nucleotide-binding domain (NBD) in BCRP. Since there are two TMD and two NBD repeats, the binding of a TMD to a

single NBD is considered as a homodimer or half-transporters. The BCRP half-transporter NBD contains a single nucleotide that binds to the TMD. BCRP exist as either homodimer or homotetramers. As part of an ATP-dependent half-transporter, BCRP becomes functional upon dimerization (Biemans-Oldehinkel et al., 2006). It excretes anthracyclines, the active metabolite of CPT-11 (SN-38), mitoxanthrone, doxorubicin, as well as many sulfate and glucuronide conjugates into bile (Ni et al., 2010; Alvarez et al., 2011). BCRP appears to be responsible for the non-Mrp2 mediated component of biliary excretion of sulfate metabolites. The role of BCRP in the biliary excretion of sulfate conjugates has been demonstrated *in vitro*, and in rat and mice organ perfusion studies (Zamek-Gliszczynski et al., 2006c; Zamek-Gliszczynski et al., 2008; Zhu et al., 2010). Negligible excretion of glutathione conjugates in Mrp2-deficient rat livers indicate BCRP's minor role in the excretion of these metabolites. BCRP transports many hydrophilic conjugated organic anions instead of hydrophobic compounds that are most likely substrates of P-gp. Working synergistically, Bcrp, Mrp-2, and P-gp eliminate a multitude of drugs absorbed across tissue barriers (Chang et al., 2011).

### BSEP.

Bile salt export pump's (BSEP, ABCB11) is another member of the Mdr family. BSEP is more than 80% homologous in human, rat, mouse, and dog. The variations are found mostly in the encoding gene of transmembrane loops (Yabuuchi et al., 2008). The exact structure of BSEP is not known (Wakabayashi et al., 2004) but it is believed that BSEP contains two trans-membrane domains composed of six helices connected between the cytoplasmic domain that is homologous to the structure of p-glycoprotein (van Den Elsen et al., 1999). However, BSEP is believed to also have four putative N-linked glycosylation sites for post-translational modifications (Borst and Elferink, 2002). BSEP is exclusively found in the canalicular membrane of the hepatocytes and has very specific substrates compared to p-glycoprotein (Kock and Brouwer, 2012). BSEP exclusively effluxes monoanionic conjugated bile acids, and whether it will excrete any other anions such as glucuronides remains to be determined. Currently, only a small number of drugs such as pravastatin and vinblastine are known to be effluxed by BSEP (Hirano et al., 2005; Yabuuchi et al., 2008). Studies have shown that deficiency in expression or function of this protein leads to intra-hepatic cholestasis and even liver injury (Balistreri et al., 2005; Hirano et al., 2006).

## 4. Possible and Likely Uptake Transporters Involved in Glucuronides Transport

Owing to their hydrophilic nature, the glucuronides also use uptake transporters, in addition to efflux transporters, to cross the biological membrane. Once formed in the intestinal epithelial cells, glucuronides are excreted into the portal vein by the efflux transporters in enterocytes, from where they enter hepatocytes with the aid of hepatic uptake transporters to be excreted into bile. The three major uptake transporter families involved in drug disposition, organic anion transporting polypeptides (OATPs), organic anion transporters (OATs), and the organic cation transporters (OCTs), likely belongs to the two solute carrier (SLC) superfamilies (Roth et al., 2012). SLC transporters are widely distributed in small intestine, kidney, liver and even brain, and are responsible for the uptake of many drugs

and/or their glucuronides. These transporters are mostly expressed on the apical membrane of enterocytes, and the basolateral membranes of hepatocytes. However, in kidney, different SLC transporters are expressed on both apical and basolateral sides (Roth et al., 2012).

The OATP family is the predominant hepatic drug uptake transporter superfamily among SLCs in liver (Vasilyeva et al., 2015). 11 isoforms of OATPs are classified into 6 families (from OATP1 to 6) and several subfamilies based on similarities in their amino acid sequences (Vasilyeva et al., 2015). OATP1B1, OATP1B3 and OATP2B1 are three of the major isoforms expressed in the hepatic basolateral membrane (van de Steeg et al., 2012; Badee et al., 2015; Rowland et al., 2015), which are responsible for most of the hepatic uptake of drugs and their metabolites.

OATP transporters show varied expression levels on the apical or basolateral side of various human tissues. OATP1B1 and 1B3 are exclusively present on the sinusoidal membrane of liver (van de Steeg et al., 2012; Vasilyeva et al., 2015), whereas OATP2B1 is ubiquitously distributed throughout human body. It is not only expressed in liver (sinusoidal membrane) but also in other organs like brain, kidney and intestine (gut lumen) (van de Steeg et al., 2012; Rowland et al., 2015). Other isoforms such as OATP1A2, OATP2A1 are expressed at a relatively low concentration. OATP1A2 is exclusively expressed in cholangiocytes, the epithelial cells of the bile duct and may be involved in the reabsorption of xenobiotics excreted into the bile. Additionally, OATP1A2 is expressed on the apical side in the intestinal epithelial cells (Roth et al., 2012). Though they participate in the uptake process, their role in drug disposition is not yet understood (van de Steeg et al., 2012; Rowland et al., 2015; Vasilyeva et al., 2015).

OATPs share a similar transmembrane domain organization, which contains 12 predicted transmembrane domains and a large fifth extracellular loop. Based on a comparison among multiple species, all OATPs/Oatps (rat) transport using a rocker-switch type of mechanism, where substrate passes through a central, positively charged pore (Roth et al., 2012; Rowland et al., 2015). OATP-mediated uptake of drugs and glucuronides is an ATP- and sodium- independent transport process, however, the exact driving force for transporters is still not clear (Roth et al., 2012). One hypothesis offered a possible explanation to the mechanism. A pharmacophore model developed for OATP1B1 base on published apparent $K_m$ values of OATP substrates suggests that substrates contain two hydrogen bond acceptors, one hydrogen bond donor and two hydrophobic regions (van de Steeg et al., 2012; Rowland et al., 2015). However, there are different mechanism among different model substrates and even different OATPs (e.g. OATP 1B1 and 1B3) have slightly different mechanism.

The pharmacological importance of OATP transporters is owed to their broad substrate specificities (Roberts et al., 2002; Roth et al., 2012). As electrogenic transporters, OATP1B1 and 1B3 facilitate transport of many drugs and their metabolites. Many anticancer drugs like irinotecan and its active metabolite SN-38, methotrexate are their substrates (Roberts et al., 2002; Kalliokoski and Niemi, 2009). Though OATP1B1 and 1B3 share most of their substrates list, OATP1B3 seems to be the only hepatic OATP responsible for the uptake of digoxin, paclitaxel and docetaxel (Kalliokoski and Niemi, 2009). Moreover, statins are also transported by OATPs (Ming, 2008; Peng et al., 2015). As the top ranked anti-

hyperlipidemia drug, statins have special importance in human health, which makes the study of OATPs more relevant and impactful. Many studies of drug–drug interactions focused on drug metabolism enzymes, such as CYPs, only. However, the wide range of the substrates as a “perpetrator” or a “victim” makes OATPs a potential target of drug-drug interactions. Pinpointing a single uptake transporter responsible for drug-drug interactions is still challenging due to overlapping substrate-specificity, which could be further impacted by the change in internal drug concentrations by metabolism enzymes.

Apart from the uptake of these exogenous substrates, OATPs are also responsible for the detoxification of endogenous compounds (Iusuf et al., 2012). For example, OATP1B1 and 1B3 play a major role in the metabolism of bilirubin and disposition of the resulting glucuronides. Together with MRP3, OATP1B1 and 1B3 establish a liver-blood shuttling loop for the transport of bilirubin and its glucuronides (van de Steeg et al., 2012; Vasilyeva et al., 2015). The functional deficiency of these two uptake transporters would lead to the blockage of the hepatocyte hopping, which causes the Rotor Syndrome (RS), a rare hereditary hyperbilirubinemia (Marin, 2012; van de Steeg et al., 2012; Rowland et al., 2015). Also, the homeostatic equilibrium of other endogenous substances such as bile acids, conjugated steroids and thyroid hormones are affected by OATPs because of their involvement in recycling of these substances (Kalliokoski and Niemi, 2009; Marin, 2012).

Organic anion transporters (OATs) are another transporter family related to the hepatic uptake. The substrates with one or two carboxylate groups are favored by the transporters (Roth et al., 2012). Though several isoforms have been detected in human liver, OAT2 is the most abundant one expressed in liver. (Jonker and Schinkel, 2004; Peng et al., 2015). Interestingly, sinusoidal membrane of hepatocyte is commonly regarded as the membrane for polarized distribution of OAT2, however, the subcellular localization of this protein in liver has only been demonstrated in rat but not in human (Jonker and Schinkel, 2004). Similar to OATPs, OAT2 is a sodium independent transporter. It works as a multi-specific organic anion exchanger by effluxing glutamate during the anion exchange. In human kidneys, OAT1, OAT2, and OAT3 are localized in the basolateral cell membrane, whereas OAT4, OAT10, and URAT1 in the apical cell membrane of proximal tubule cells, respectively. Studies have shown that OAT1 and OAT3 are involved in the elimination of various classes of drugs and conjugates of endogenous and exogenous substances from blood to urine in renal proximal tubules (Emami Riedmaier et al., 2012; Roth et al., 2012).

Compared to OAT1 and OAT3, a relatively small number of drugs are transported by OAT2. Some antiviral drugs, ACE inhibitors, angiotensin II receptor antagonists, HMG-CoA reductase inhibitors, NSAIDs, and antitumor drugs (paclitaxel, methotrexate etc.) are its substrates (Jonker and Schinkel, 2004; Estudante et al., 2013). In addition, a number of endogenous substances including sulfated steroid hormones (estrone-3-sulphate etc.), second messengers (Cyclic GMP and Cyclic AMP) and nucleosides (adenine, cytidine, guanosine etc.) are transported by OAT2 (Jonker and Schinkel, 2004).

## 5. Driving Forces for the Systemic Distribution of Metabolites

### Polarized Excretion of Glucuronides.

In major metabolic organs such as intestine, liver and kidney, the primary cells responsible for metabolism are polarized, and as such efflux transporters located on the basolateral membrane are mostly distinct from those located on the apical membrane. Assuming stable UGT expression and functionality, transporters located at the basolateral membrane of enterocytes and hepatocytes, function as the driving forces for glucuronides to be distributed into blood. On the other hand, hepatic uptake transporters are able to extract extra-hepatic generated glucuronides, limiting their systemic exposure and facilitating their elimination via biliary excretion.

### Role of Basolateral Efflux Transporters.

MRP3 is an important transporter located at the basolateral side of enterocytes and sinusoidal membrane of hepatocytes. Various glucuronide conjugates have been identified as substrates of MRP3, and MRP3 facilitates their entry into the mesenteric blood or the general circulation. In vesicular transport and ATPase activity assays, it was shown that estradiol 17-β-d-glucuronide, 7-hydroxycoumarin glucuronide, morphine glucuronide and bisphenol A glucuronide are substrates of MRP3 (Zelcer et al., 2001; Zamek-Gliszczynski et al., 2011; Mazur et al., 2012; Wittgen et al., 2012). Studies in everted intestinal sacs showed that the serosal efflux rate of 4-methylumbelliferone glucuronide decreased significantly in the small intestine of Mrp3 knockout mice (Kitamura et al., 2010). In Mrp3 knockout mice, the levels of resveratrol-3-glucuronide were up to 10-fold lower in plasma and urine (van de Wetering et al., 2009), and levels of morphine-3-glucuronide were up to 50-fold lower in plasma (Zelcer et al., 2005), owing to the lack of Mrp3 in the basolateral membrane of the enterocytes. In addition to MRP3, MRP4 is responsible for driving the intracellular glucuronides into the circulation from hepatocytes. In sandwich-cultured human hepatocytes and membrane vesicle uptake assays, the involvement of MRP3 and MRP4 in hepatic transport of mycophenolic acid glucuronide into the circulation was reported (Matsunaga et al., 2014).

Apical efflux transporters do not directly contribute towards the systemic exposure of glucuronides, however they can affect the outcome indirectly. The reduce expression of apical efflux transporter MRP2 is often associated with up-regulation of basolateral efflux transporter MRP3 to limit hepatic toxicity (Keppler and Konig, 2000; Dietrich et al., 2001; Roberts et al., 2002; Kubo et al., 2009). Owing to this, the systemic exposure of endogenous substrates and xenobiotic can be greatly altered due to the altered expression level or inhibition of apical efflux transporters.

### Role of Basolateral Uptake Transporters.

When the extra-hepatic generated glucuronides reach portal vein, they have a chance to be taken up by the hepatocytes. OATP transporters expressed on the sinusoidal membrane of the hepatocytes play an important role in hepatic uptake of many endogenous and exogenous conjugates. For instance, studies in Oatp1a/1b knockout mice suggested that Oatp1a/1b transporters are involved in hepatic uptake of glucuronidated bilirubin (van de Steeg et al.,

2010). There is also evidence showing that OATP1 plays important role in mediating the uptake of estradiol 17β-d-glucuronide by hepatocytes (Kouzuki et al., 1999). Moreover, OATP2B1 was demonstrated to be a major transporter involved in hepatic uptake of scutellarein glucuronides, which was suggested as a predominant process for their pharmacokinetic behaviors (Gao et al., 2012). Daidzein-7- glucuronide was reported as a substrate of OATP2B1 as well (Grosser et al., 2015). More detailed discussions can be found in Section 4.

In the liver, the presence of uptake transporters (e.g. OATPs) provides a potential pathway for extrahepatically generated glucuronides to be excreted into bile and therefore enhances the enterohepatic circulation of glucuronide conjugates. The circulation half-life of a glucuronide can be prolonged as the result of the increased enterohepatic recycling. On the other hand, the OATP uptake transporters also work in concert with sinusoidal export transporters (e.g.MRP3). Van de Steeg et al. proposed a theory they called “hepatocyte hopping” based on their observations with glucuronidated bilirubin in Oatp1a/1b mice. They found that the plasma level of bilirubin glucuronide in the Oatp1a/1b-deficient mice is remarkably increased. It was hypothesized that in the presence of both Oatp1a/1b and Mrp3 transporters, bilirubin glucuronide secreted in blood by Mrp3 can be taken up again by Oatp1a/1b located in the neighboring hepatocytes and hence have another chance to be excreted into bile. They claimed that this hepatocyte shuttling process can efficiently prevent the buildup of bilirubin glucuronide in the circulation (van de Steeg et al., 2010; Iusuf et al., 2012).

An important contributor to the systemic distribution of metabolites is recycling of glucuronides that are eliminated via bile or directly into the intestinal lumen. Because of the presence of microflora, glucuronides can be reconverted back into aglycone, which can then be reabsorbed, completing the recycling loop. More detailed discussion can be found in Section 6 below.

## 6. Recycling Mechanisms

Recycling prolongs the exposure of drugs to the systemic circulation due to repeated hydrolysis of glucuronides by (microbial) β-glucuronidase in gut, followed by reabsorption of the parent compound. Endogenous and exogenous substances such as xenobiotics and environmental pollutants can participate in one or more of the three types of recycling processes; enterohepatic, enteric and local recycling. Many compounds undergo only enterohepatic recycling (Gao et al., 2014) but some compounds such as polyphenols undergo duo recycling scheme involving both enteric and enterohepatic recycling (Roberts et al., 2002; Chen et al., 2003a; Liu et al., 2003; Jia et al., 2004; Silberberg et al., 2006; Liu and Hu, 2007). Similarly, local recycling could also occur independently or along with enteric and enterohepatic recycling, depending on the drug disposition characteristics. It is suggested that the relative contribution of enterohepatic, enteric and local recycling in the overall disposition of drug depends on the efficiency of enzyme-transporter coupling (see Section 8) by controlling the amounts of metabolites excreted by the intestine and liver (Jia et al., 2004; Jeong et al., 2005b; Liu and Hu, 2007).

### Enterohepatic Recycling.

Enterohepatic recycling also known as enterohepatic circulation is a process where certain drug absorbed by enterocytes reenters intestine via bile excretion as glucuronides, which upon hydrolyzing back to aglycone, are reabsorbed. The drug once absorbed by intestinal cells enters the portal vein by the passive diffusion, whereas its glucuronides formed in the intestinal cell excrete on the basolateral side (gut lumen) by the efflux transporters. The drug from portal vein then enters hepatocytes by passive diffusion, where it can get metabolized again. On the other hand, glucuronides in portal vein may be taken up by the hepatocytes with the aid of hepatic sinusoidal uptake transporters. Hepatic efflux transporters on the apical side (MRP2 and BCRP) then can return the glucuronide to the intestine via the bile duct (Roberts et al., 2002; Gao et al., 2014) (Fig 2).

The bile contains glucuronides that are emptied into the gut after a meal, where glucuronides are hydrolyzed into parent drug by gut microflora followed by reabsorption in colon, thereby entering enterohepatic recycling (Roberts et al., 2002; Chen et al., 2003a; Liu et al., 2003; Jia et al., 2004; Silberberg et al., 2006; Liu and Hu, 2007). This process repeats itself until the drug is eliminated from the body. Enterohepatic recycling increases the half-life and the residence time for the species being recycled, and thus increase systemic exposure and delay drug clearance, as evident by the prolonged terminal elimination phase (Ouellet and Pollack, 1995; Schaiquevich et al., 2002). A drug undergoing enterohepatic recycling usually shows the multiple-peak phenomenon in its plasma-concentration–time profile and the prolonged elimination half-life (Gao et al., 2014). Ezetimibe (Ezzet et al., 2001; Yamamoto et al., 2007), sorafenib (Vasilyeva et al., 2015), diclofenac (Fukuyama et al., 1994), irinotecan (and SN-38) (Younis et al., 2009) and morphine (Ouellet and Pollack, 1995) are some of the drugs, which have shown to undergo biliary excretion and enterohepatic recycling as glucuronides in various animal models.

The enterohepatic recycling of glucuronides can have either beneficial or harmful effects on the body. In certain cases, such as flavonoids with lower bioavailability due to high metabolic clearance, the recycling increases their systemic exposure, half-life and the residence time in body, which is a favorable outcome (Hu, 2007; Thilakarathna and Rupasinghe, 2013; Dai et al., 2015). However, in other cases, this process is an unwanted outcome. For example, the enterohepatic recycling of diclofenac acyl glucuronides could increase the potential of gut toxicity by the repeated exposure to NSAID (Seitz and Boelsterli, 1998). Similarly, deconjugation of SN-38-glucuronide by gut microflora, results in high concentrations of SN-38 locally, thereby causing severe delayed diarrhea (Kaneda et al., 1990).

### Enteric Recycling.

Certain percentages of parent drug that enter intestinal cells after oral administration are metabolized into glucuronides(s). These glucuronides can either enter the mesenteric blood system by the basolateral efflux transporters, or effluxed back into intestinal lumen by the apical efflux transporter(s). In the gut lumen, glucuronides are not absorbed and they will travel down the intestine until they reach terminal ileum or colon (bacteria-rich regions of the gut), where they are converted back into the parent drug by the gut microflora, and

reabsorbed, thereby completing the enteric recycling scheme. A compound may be recycled repeatedly until it is completely eliminated from the system (Fig 2).

Multiple studies have been published to delineate the role of various components of enteric recycling scheme of glucuronides (UGT and efflux transporters) and understand how these components can be modulated to affect the local and systemic bioavailability of compounds/aglycones undergoing glucuronidation. A combination of *in situ* rat/mouse intestinal perfusion model along with *in vitro* intestinal/hepatic microsomes and Caco-2 cell transport studies demonstrated that enteric recycling plays an important role in disposition of various flavonoids, owing to their extensive glucuronidation in intestine and excretion of these glucuronides in gut lumen (Chen et al., 2003a; Hu et al., 2003; Liu et al., 2003; Jia et al., 2004; Chen et al., 2005a; Jeong et al., 2005b; Wang et al., 2006). MK-571 (Mrp2 inhibitor) and dipyridamole (BCRP inhibitor) when used together were able to significantly decrease the intestinal and biliary excretion of naringenin glucuronides in Wistar rats. These findings strongly suggested the involvement of MRP2 and BCRP efflux transporters in the enterohepatic and enteric recycling, by controlling the biliary and luminal efflux of glucuronides in liver and intestine, respectively (Xu et al., 2009).

Contrary to enterohepatic recycling, excretion of conjugates by enterocytes in enteric recycling do not usually cause double peak phenomenon, mainly because metabolites are gradually and continuously excreted into large intestine to be hydrolyzed and reabsorbed (Roberts et al., 2002; Chen et al., 2003a; Liu et al., 2003; Jia et al., 2004; Silberberg et al., 2006; Liu and Hu, 2007). In the enteric recycling of glucuronides, action of both, UGT enzyme (present at higher levels in the small intestine) and microbial β-glucuronidases (present at higher levels in the large intestine), are required and recycling is completed over the entire intestine. Moreover, enteric recycling does not require hepatic enzymes and efflux transporters observed for enterohepatic recycling, but rely only on the intestinal enzymes and efflux transporters.

### Local Recycling.

More recently, a novel recycling system called local recycling of glucuronide has been reported, where drug enters the recycling mechanism without the intervention of bacterial β-glucuronidases. In local recycling, the deconjugation of glucuronides into parent drug is carried out by β-glucuronidases of enterocytes in upper small intestine, followed by reabsorption of drug in lower part of gut (Fig 2) (Xia et al., 2012; Dai et al., 2015). Wogonoside was rapidly hydrolyzed into wogonin by the β-glucuronidase present in the enterocytes rather than that of gut lumen (Xia et al., 2012). Dai et al. showed that tilianin could enter enteric, enterohepatic and local recycling scheme, called the triple-recycling mechanisms, after metabolizing into three metabolites; tilianin glucuronide, acacetin, and acacetin glucuronide (Dai et al., 2015).

Local recycling prolongs the residence time and increase local exposure of flavonoids in the gut and thus, it is assumed that flavonoids may have more biological activities in the gut than predicted based on their poor systemic bioavailability (Jeong et al., 2005a; Hu, 2007; Zhang et al., 2007; Xia et al., 2012). Though the local recycling has been reported in only two instances so far (Xia et al., 2012; Dai et al., 2015), based on the proposed mechanism of

action, it is very much possible that local recycling significantly affects the biological activities of other drugs, which are undergoing extensive glucuronidation in gut. Local recycling of glucuronides, along with their enteric and enterohepatic recycling, can lead to prolonged and higher systemic exposure of poorly bioavailable phenolics, both locally and systemically. This prospect is equally important in the case of locally active drugs such as ezetimibe and ezetimibe glucuronide (more active than the parent compound), which exerts their cholesterol-lowering action by reducing the uptake and absorption of cholesterol by enterocytes. Possible triple recycling of ezetimibe glucuronide leads to prolonged local exposure in gut, resulting in more bioactivity at the site of action (Kosoglou et al., 2005). Similarly, SN-38 toxicity in large intestine is attributed to deconjugation of SN-38 glucuronide to toxic aglycone by bacteria β-glucuronidase in colon (Wallace et al., 2010). However, upper small intestine toxicity may be due to the local recycling, but this has not been investigated fully yet.

Like enteric recycling, local recycling requires only intestinal enzymes and transporters, and does not exhibit any double peak phenomenon, as the excretion of glucuronide is continuous. On the other hand, unlike the enteric recycling, local recycling could complete in the small intestine alone, without involving the whole intestine, as the action of microbial β-glucuronidases is not needed. Local recycling can occur either independently or in conjugation with other recycling processes. For drugs that are extensively metabolized in the gut, glucuronides can be excreted in gut lumen from enterocytes where they can re-enter the intestine after hydrolyzing back to aglycone by β-glucuronidases of enterocytes, thereby completing the cycle without the involvement of the other recycling mechanisms. However, rapidly absorbing drugs can saturate the gut UGT at high concentrations, so that they bypass intestinal metabolism and are predominantly glucuronidated in the liver. For these compounds, local recycling does not play significant role in first pass metabolism, but the glucuronides can later participate in local recycling followed by excretion in lumen through bile (Xia et al., 2012).

## 7. Driving Forces for the Elimination of Glucuronides

### Routes of Elimination.

The elimination of glucuronides includes biliary, urinary, and intestinal excretion. For example, it has been shown that the biliary, urinary, and intestinal excretion of acetaminophen glucuronide accounts for 13, 9, and 1% of the orally administered acetaminophen in rats (Villanueva et al., 2008). Because of their involvement in the excretion of hydrophilic glucuronides, various membrane transporters (efflux and uptake) together determine the preferential elimination pathway for the disposition of glucuronides.

### Intestinal Excretion.

In enterocytes, MRP2 and BCRP are the apical transporters, which have been shown to mediate the efflux of intracellular-formed glucuronide metabolites into the lumen (Fig 1a). In the lumen, the glucuronides can either be excreted in the feces or hydrolyzed back to the aglycone. For example, in Caco-2 cells, there was about one-fold decrease in the apical efflux of hesperetin glucuronide when Ko143 (5 μM) was used as BCRP inhibitor (Brand et

al., 2008). Similarly, the use of LTC4 (an inhibitor of MRP2) decreased the efflux of emodin glucuronide from basolateral to apical side significantly (Liu et al., 2012a). The efflux of luteolin glucuronides from HeLa cells overexpressing UGT1A9 was inhibited by Ko143 in a dose-dependent manner (Tang et al., 2014). In rat perfusion model, Mrp2 and Bcrp1 were shown to efflux naringenin glucuronide to intestine and compensate for each other (Xu et al., 2009). As reported by our group, in Bcrp1 knockout mice, the excretion rate of genistein glucuronide in the small intestine decreased significantly (78%) (Zhu et al., 2010), whereas in Bcrp1-defecient mice, a substantial increase (>10 folds) in plasma AUC of genistein glucuronide after oral dose of genistein was observed (Yang et al., 2012). Similarly, in bioavailability and tissue distribution studies of resveratrol in Bcrp1 knockout mice, it was shown that Bcrp1 mediated the efflux of resveratrol glucuronides to the intestinal lumen and the AUC of resveratrol glucuronides increased in Bcrp1-deficient mice (Alfaras et al., 2010).

### Hepatocyte Excretion.

In hepatocytes, MRP2 and BCRP are located on the canalicular membrane, where they function as efflux pumps to move intracellular glucuronides into bile (Fig 1b). The presence of these transporters provides a pathway for hepatic excretion and facilitates enterohepatic recycling. In Mrp2 knockout mice, a 56% decrease in biliary excretion of ezetimibe glucuronide was observed (de Waart et al., 2009), while the serum ezetimibe glucuronide levels in Mrp2-deficient rat increased by 10 folds compared to that in wild-type rats (de Waart et al., 2009; Oswald et al., 2010). Moreover, in the intestinal and liver perfusion studies in Mrp2-deficient rats, the excretion of ethinylestradiol glucuronide into intestine and bile was significantly deceased, and the systemic exposure of ethinylestradiol glucuronide was 46-fold higher in Mrp-2 knockout mice due to its decreased excretion into lumen and bile (Zamek-Gliszczynski et al., 2011). Similarly, Mrp2 has also been reported to mediate the biliary excretion of mycophenolic acid-7-O-glucuronide, 4-methylumbelliferyl glucuronide, flavopiridol glucuronide, grepafloxacin glucuronide, 17β-estradiol-17-β-D-glucuronide, and resveratrol glucuronide (Sasabe et al., 1998; Morikawa et al., 2000; Jager et al., 2003b; Jager et al., 2003a; Westley et al., 2006; Zamek-Gliszczynski et al., 2006a; Maier-Salamon et al., 2008). In *in situ* perfusion studies in Bcrp1(−/−) and Mrp2 (−/−) mouse livers, Bcrp was demonstrated to play a major role in biliary excretion of glucuronide metabolites of acetaminophen, 4-methylumbelliferone, and harmol, whereas Mrp2 played only a minor role (Zamek-Gliszczynski et al., 2006c). In efflux transporter-deficient mice, it was shown that both MRP2 and BCRP mediate the biliary excretion of droxydiclofenac acyl glucuronide (Lagas et al., 2010). Since, MRP2 and BCRP have overlapping substrate specificities, it is challenging to predict the impact of altered function of one or more transporter on the biliary excretion and systemic exposure of glucuronides (Yang et al., 2014).

### Kidney Excretion.

In hepatocytes, glucuronides are either excreted by apical efflux transporters such as MRP2 and BCRP into bile, or they enter the systemic circulation by basolateral efflux transporters such as MRP3 (Fig 1c). From systemic circulation, glucuronides can be taken up into the kidney proximal tubular cells via organic anion transporters to be excreted via MRP2 or MRP4 at the apical side of these cells. Several transporters haven been shown to mediate the

renal handling of glucuronides on the apical and basolateral sides of renal epithelial cells. Glomerular Filtration Rate (GRF) is also an important factor for the urinary excretion of glucuronides. For example, it had been shown that mycophenolic acid glucuronide (MPAG) and morphine glucuronides levels were higher in patients with poor renal function because of the decreased renal clearance of these glucuronide metabolites (Osborne et al., 1993; Naesens et al., 2007).

Organic anion transporters (OATs) expressed on the basolateral membrane of the proximal tubules in kidney are known to be involved in the transport of glucuronides. Studies in human embryonic kidney 293 (HEK 293) cells overexpressing hOAT3 showed that there was an enhanced uptake of MPAG (Uwai et al., 2007), morinidazole glucuronides (Zhong et al., 2014), daidzein-7-O-glucuronide, genistein-7-O-glucuronide, glycitein-7-O-glucuronide and quercetin-3-O-glucuronide (Wong et al., 2011), which suggested that OAT3 contributes to the renal tubular secretion of these glucuronide conjugates.

For renally excreted drugs, MRP2 and MRP4 plays a significantly role in the excretion of glucuronide in a substrate-dependent manner. On the apical side of renal cells, it was demonstrated that MRP4 was involved in the urinary excretion of glucuronide metabolite of edaravone, and the renal excretion of edaravone glucuronide was 2-fold lower in Mrp4-deficient mice (Mizuno et al., 2007). The role of MRP2 and MRP4 in renal excretion of MPAG was studied in HEK293 cells overexpressing human transporters and in isolated perfused kidneys. It was found that MPAG was a substrate of MRP2 but not MRP4, and the urinary excretion of MPAG was significantly greater in wild-type rat kidneys than in Mrp2 (−/−) rat kidneys (El-Sheikh et al., 2014). It was also reported that the renal excretion of acetaminophen glucuronide increased by 200% in bile duct-ligated rats, and this alteration was attributed to the up-regulation of renal Mrp2 (Villanueva et al., 2008). The glucuronide of 7-hydroxycoumarin (7-HC-G) was shown to be a substrate of MRP4 in studies using membrane vesicles overexpressing MRP transporters, and MRP4 was suggested to play a role in the excretion of 7-HC-G in kidney (Wittgen et al., 2012).

## 8. Interplay of UGT Enzymes, Efflux and Uptake Transporters

The disposition of drugs undergoing glucuronidation is usually controlled by multiple serial and/or parallel processes. First the drug is absorbed into enterocytes via passive diffusion, where it converts to glucuronides by intestinal UGTs. The gut-generated glucuronides are then excreted into gut lumen and portal vein by apical and basolateral intestinal efflux transporters, respectively. From portal vein, drug enter hepatocytes by passive diffusion and undergo glucuronidation by hepatic UGTs, whereas, glucuronides are taken up by the hepatic uptake transporters. From hepatocytes, both intestinal- and hepatic-generated glucuronides are then excreted into bile and systemic circulation by the apical and basolateral hepatic efflux transporters, respectively. These steps are further inter-linked with the three recycling mechanism mentioned in section 6.

In such a multi-component system, interplay between two or more components and processes is highly probable. The interplay could be explained by a combination of variety of coupling mechanisms for the disposition of glucuronides: UGT enzyme-efflux transporter

coupling; UGT-uptake transporter coupling; efflux-uptake transporters coupling; and UGT enzyme-efflux transporter-uptake transporters coupling. These coupling mechanisms with the participation of multiple enzyme isoforms, efflux transporters and uptake transporters create a multi-component Enzyme–Transporter coupling network. Despite its complexity, this coupling network is highly capable of protecting human body against exogenous toxins; maintaining homeostasis of endogenous chemicals; and acting as bioavailability barrier to many xenobiotics. Importantly, failure in any of the individual components of the network is highly unlikely to cause the failure of the entire network.

### UGT Enzyme–Efflux Transporter Coupling.

In the disposition of the drugs undergoing glucuronidation, the process of glucuronidation by UGTs couples with the process of excretion of glucuronides by efflux transporters. This phenomenon can be explained by the “Revolving door theory”, where the efflux transporters act as “revolving door” to facilitate and/or control the excretion of hydrophilic glucuronides out of the liver and intestinal cells (Liu and Hu, 2007; Singh and Hu, 2011). The UGT-efflux transporter coupling can leads to an imbalance between formation and excretion of glucuronides if one of the above processes act as the rate-limiting step. As a result, the actual rate of glucuronide excretion could either be lower (in efflux rate –limiting) or higher (in UGT rate-limiting) than the estimated rate of glucuronide excretion based on the cellular UGT activity (as measured from subcellular fraction) (Chen et al., 2003a; Jia et al., 2004; Jeong et al., 2005b; Wang et al., 2006).

Multiple UGT isoforms and efflux transporters (such as MRP2, MRP3 and BCRP) are shown to participates in the coupling process (Zamek-Gliszczynski et al., 2006b; Zhou et al., 2010; Jiang et al., 2012; Yang et al., 2012; Wei et al., 2013; Tang et al., 2014; Zhang et al., 2015; Wang et al., 2016). For example, estradiol-17-beta-d-glucuronide, which is formed by UGTs 1A1, 1A3, 1A4, 1A8, 1A9, and 1A10, was shown to interact with MRP2 and MRP3, expressed in isolated Sf9 membrane vesicles (Bodo et al., 2003). Also, enzyme-transporter interplay was observed in genistein glucuronide excretion in Hela cell overexpressed with UGT1A9 and BCRP. Ko143, a potent BCRP inhibitor was able to reduce the clearance of genistein glucuronide by about 75–94% in a dose-dependent manner (Jiang et al., 2012). Similarly, MK-571, a non-specific chemical inhibitor of MRP2, MRP3, and MRP4, significantly reduced the efflux of emodine glucuronide in the apical-to-basolateral (A-B) and B-A directions in Caco-2 cell lines in a dose-dependent manner (Liu et al., 2012a). Emodine is majorly glucuronidated by UGT1A1, 1A9, 1A10 and 2B7 (Wu et al., 2014). Due to overlapping substrate-specificity (Tian et al., 2008; Zhou et al., 2010; Singh et al., 2011a; Keppler, 2014; Yang et al., 2014) among UGT isoforms and efflux transporters, in an event of inhibition or deficiency of a UGT isoform or efflux transporters, other isoforms and transporters from same family and/or sub-family compensate for them (Wang et al., 2009; Xu et al., 2009). This mechanism makes it highly difficult to delineate individual UGT Enzyme-Efflux Transporter coupling pairs.

Specific inhibitors for various UGT isoforms and efflux transporter participating in the network are not readily available, so that the importance of individual enzyme isoform and transporter in the network has not been determined so far. Therefore, it has not yet been

possible to overcome the bioavailability barrier by targeting a specific UGT enzyme(s) and/or an efflux transporter(s) of glucuronides. Furthermore, it is challenging to study this compensation mechanism, as when gene(s) of interest is silenced or induced for prolonged period of time, the expression of other enzymes and transporters of same family or subfamily are up- or down-regulated, respectively (Johnson et al., 2006; Hoffmann and Loscher, 2007; Kubo et al., 2009; Miyawaki et al., 2012).

### UGT Enzyme-efflux transporter-uptake transporters coupling.

Once a glucuronide is excreted into gut lumen or the portal vein by efflux transporters in enterocytes, the uptake transporters in hepatocytes and enterocytes could take it up from portal vein and intestinal lumen, respectively. Due to overlapping substrate-specificity between various efflux and uptake transporters of glucuronides (Liu et al., 2006; Kalliokoski and Niemi, 2009; Kindla et al., 2009; Fahrmayr et al., 2010; Keppler, 2014), it could be hypothesized that the net excretion of glucuronides from enterocyte and hepatocyte could be dependent both on uptake of glucuronides into the cell by uptake transporters (such as OATPs and OATs) and efflux of glucuronides out of the cell by efflux transporters (such as MRPs and BCRP). The glucuronide taken up in the process can further be excreted into bile by hepatocytes or portal vein by enterocytes. The glucuronide excreted into the intestinal lumen and bile (emptying into gut) from apical efflux transporters of enterocytes and hepatocytes, respectively, can be reabsorbed after getting deconjugated in intestine thereby entering the recycling mechanisms (see Section 6).

UGT Enzyme-efflux transporter interplay has been investigated extensively in last decade, however, other coupling mechanisms with respect to xenobiotic glucuronidation are yet to be explored in depth. Though such couplings have been successfully shown for CYP substrates and endogenous glucuronides (Nies et al., 2004; van de Steeg et al., 2010; Iusuf et al., 2012; van de Steeg et al., 2012; Daali et al., 2013; Neve et al., 2013; Li et al., 2014; Shi and Li, 2014; Vasilyeva et al., 2015), very little has been reported for UGT substrates. OATP1B1 has been shown to play crucial role in the hepatic transport of glucuronides. Gemfibrozil glucuronide was able to inhibit the OATP1B1- and OATP1B3-mediated hepatic uptake of pravastatin (Nakagomi-Hagihara et al., 2007a; Nakagomi-Hagihara et al., 2007b). Uptake of ezetimibe glucuronide in cell expressing OATP1B1*1b was reduced as compared to the uptake in cell expressing wild-type protein (Oswald et al., 2008). Similarly, pharmacokinetics of ezetimibe glucuronide in human subjects with OATP1B1 polymorphism was affected. Fecal ezetimibe glucuronide excretion was significantly decreased whereas renal glucuronide excretion was increased in carriers of *1b/*1b. Polymorphism of OAT1B1 affect the uptake of ezetimibe glucuronide from portal vein into hepatocyte, such that reduced levels of ezetimibe glucuronides are available in hepatocytes for biliary excretion. However, this did not cause the expected increase in systemic concentration of ezetimibe glucuronides, probably due to increased renal clearance of glucuronides (Oswald et al., 2008). Very recently, sorafenib-glucuronide has been shown to display in UGT enzyme - efflux transporter (MRP2/MRP3) - uptake transporter (OAPT1B1/1B3) coupling, hepatocyte shuttling/hopping, as well as enterohepatic recycling (Vasilyeva et al., 2015). However, further mechanistic studies are required to understand these mechanisms and their implications in clinical drug-drug interactions.

## 9. New Directions

An important area, which is gaining significant attention and requires further exploration is the effect of gut microbiome on the disposition of drugs undergoing glucuronidation through enteric and enterohepatic recycling mechanism. Diet and antibiotics drugs can significant alter the microbial population in gut, thereby influencing the drug systemic exposure and disposition by affecting its re-entry into systemic circulation. Moreover, gut microbiome can also be used as therapeutic target to reduce drug related gut and liver toxicity owing to enterohepatic recycling. SN-38-glucuronide (phase-II metabolite of CPT-11) is converted into SN-38 (phase-I metabolite of CPT-11) by β-glucuronidase present in gut microflora, resulting in high luminal concentrations of SN-38, thereby causing severe delayed diarrhea (Kaneda et al., 1990). Potent bacterial β-glucuronidase inhibitors (1, 2, 3, and 4) (Fig 3) with submicromolar $IC_{50}$ and $K_i$ values have been identified recently that can block the conversion of glucuronides to aglycone, thereby blocking the enterohepatic recirculation of CPT-11 and NSAIDs. Crystal structures of E Coli β-glucuronidase complexes with inhibitors showed that inhibitors were bound at the “bacterial loops” at the entrance to the active-site cavity. Inhibitor 1 was shown to significantly reduce diarrhea and lower GI damage in 6- to 8-week-old Balb/cJ mouse models of CPT-11-induced toxicity (Wallace et al., 2010; LoGuidice et al., 2012).

## 10. Summary

The presence of multiple driving forces makes the disposition of drugs via glucuronidation process very complex in nature, when comparing to drug disposition via CYPs. Atypical behavior in glucuronidation is often observed when two drugs interact via a phase II disposition mechanism. For example, for drug interaction via phase I enzymatic inhibition, substrate levels in plasma increases but metabolite levels decreases (Dresser et al., 2000; Stearns et al., 2003; Laugesen et al., 2005). For a drug that is a substrate of both CYP and p-glycoprotein, inhibition of the efflux transporter led to higher concentration of both the metabolite and the corresponding aglycone (Pang et al., 2009; Li et al., 2014). In contrast, for drugs undergoing phase II metabolism, a drug interaction via efflux transporter inhibition could lead to higher plasma levels of metabolite with or without a corresponding increase in the aglycone levels, even though the enzyme activities were not altered (Yang et al., 2012; Wei et al., 2013; Ge et al., 2016). This complex process is also the reason why *in vitro* glucuronidation obtained from organ microsomes often cannot predict *in vivo* glucuronide production or levels of glucuronides in plasma (Wang et al., 2006; Wu et al., 2013).

The complex process involves the interplay of various enzyme and transporter systems including recycling mechanisms to control the system exposure and clearance of these drugs. The interplay can happen between enzyme system (UGT1A and UGT2B) and efflux transporters (both apical and basolateral) in enterocytes and hepatocytes as well as between the efflux and uptake transporters of hepatocytes (Liu and Hu, 2007; Jiang and Hu, 2012; Kock and Brouwer, 2012; Wu, 2012; Pfeifer et al., 2014; Zamek-Gliszczynski et al., 2014). Such a complex interplay of various components is possibly essential for the body to maintain a tight control over the disposition of various endogenous compounds such as bile

acid, bilirubin and steroids in order to maintain their homeostasis, as well as the detoxification of environmental and dietary toxins.

The absence or inhibition of a particular component in this complex equation can cause compensation by one or more components to avoid cell toxicity, thereby resulting in increased or decreased concentration of parent compound or the glucuronide in the systemic circulation. For e.g., MRP3 up-regulation in event of MRP2-deficiency can cause increased bilirubin glucuronide excretion in blood (Kamisako et al., 2000). Similarly, comparable or even higher level of glucuronidation by Ugt1a-deficient Gunn rats as compared to the control Wistar rats, were ascribed to the compensatory up-regulation of intestinal Ugt2bs and hepatic anion efflux transporters (Wang et al., 2009).

However, the downside of this complex system is a very difficult-to-overcome oral bioavailability barrier for xenobiotics using glucuronidation as major elimination pathway (Hu, 2007; Gao and Hu, 2010). Many recent studies have been published showing how these individual components contribute to the overall disposition mechanism, as well as how the modulation of one or more these components can alter the systemic exposure of glucuronidated compounds (Wei et al., 2013; Tang et al., 2014; Dai et al., 2015; Zhang et al., 2015; Wang et al., 2016; Zeng et al., 2016). The published research with UGT- or transporter- deficient or over-expressed animal or cell models indicates that it is very difficult to improve bioavailability of the drug by interfering with one or the other components. However, the systemic exposure can be modulated by altering the excretion of glucuronide in blood by inhibiting the one or more efflux and/or uptake transporters. Further detailed studies to understand the interplay of various components will be needed in order to improve the systemic and/or local exposure of beneficial UGT substrates.

In conclusion, systemic glucuronide levels are often not determined by the UGT enzyme activities alone but also by the action of efflux transporters that mediate the distribution of glucuronides into the systemic circulation. This mechanism means that any drug interaction involving an efflux transporter of glucuronides can have a direct impact on the systemic levels of the glucuronides, which in turn could change the levels of their corresponding aglycone due to the presence of glucuronidases. On the other hand, the systemic clearance of glucuronides is also affected by the recycling of substrates, which undergo glucuronidation via local, enteric and enterohepatic recycling (i.e., so called triple-recycling mechanisms). Hence, predicting systemic glucuronide levels requires the consideration of the structure and function of intestinal microbiome. Taken together, recent advances in understanding the glucuronidation process will help us improve the systemic and local bioavailability of drugs that undergo phase II glucuronidation.

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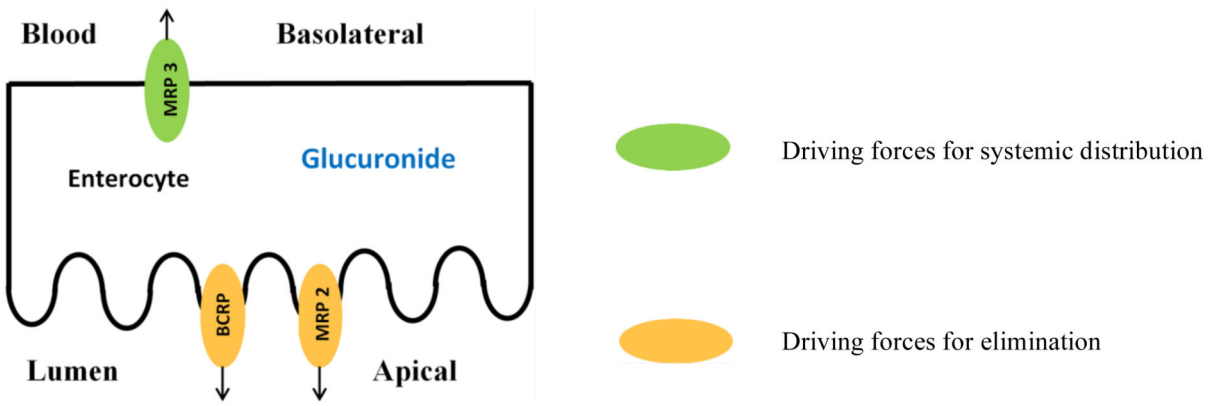
oroxylin A by human liver and intestinal microsomes. Pharm Res 27:1568–1583. [PubMed: 20411407]

Zhou X, Li L, Deng P, Chen X, and Zhong D (2013) Characterization of metabolites of GLS4 in humans using ultrahigh-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 27:2483–2492. [PubMed: 24097405]

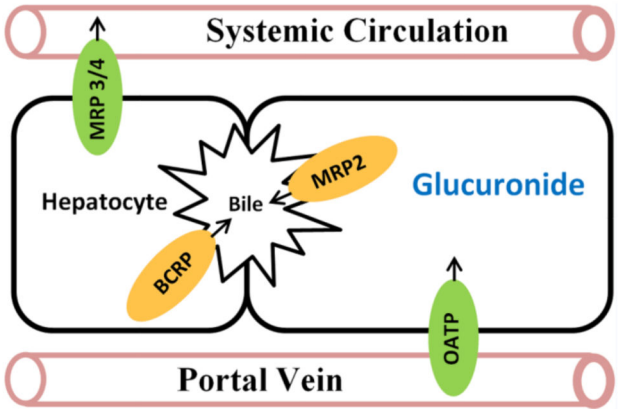
Zhu W, Xu H, Wang SW, and Hu M (2010) Breast cancer resistance protein (BCRP) and sulfotransferases contribute significantly to the disposition of genistein in mouse intestine. Aaps J 12:525–536. [PubMed: 20582579]

Zhu Y, Li L, Deng P, Chen X, and Zhong D (2016) Characterization of TPN729 metabolites in humans using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. J Pharm Biomed Anal 117:217–226. [PubMed: 26366939]

(a)




(b)



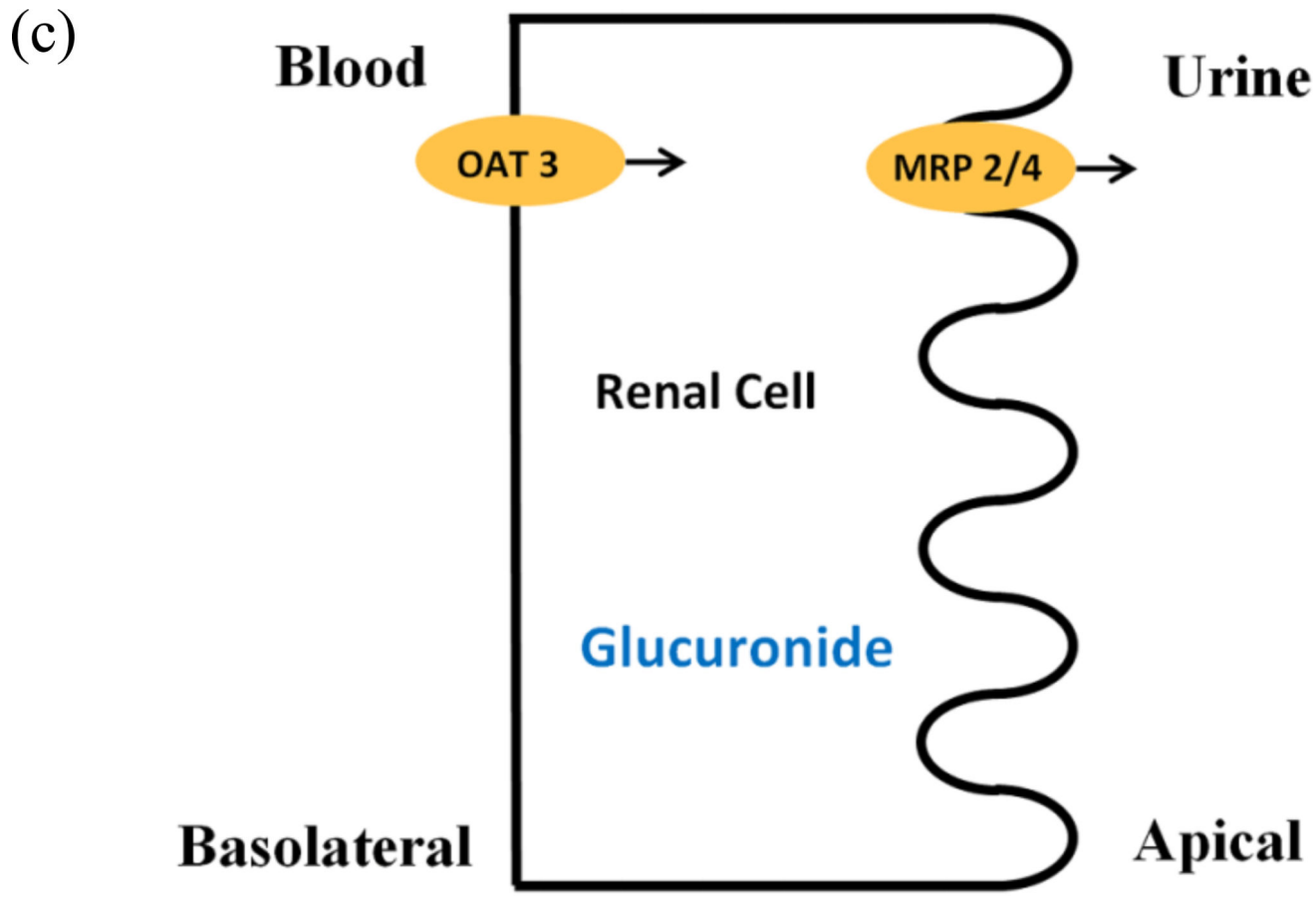



**Figure 1.**
Graphs illustrating the driving forces for systemic distribution (green) and elimination (orange) of glucuronide in enterocytes (a), hepatocytes (b), and renal cells (c).

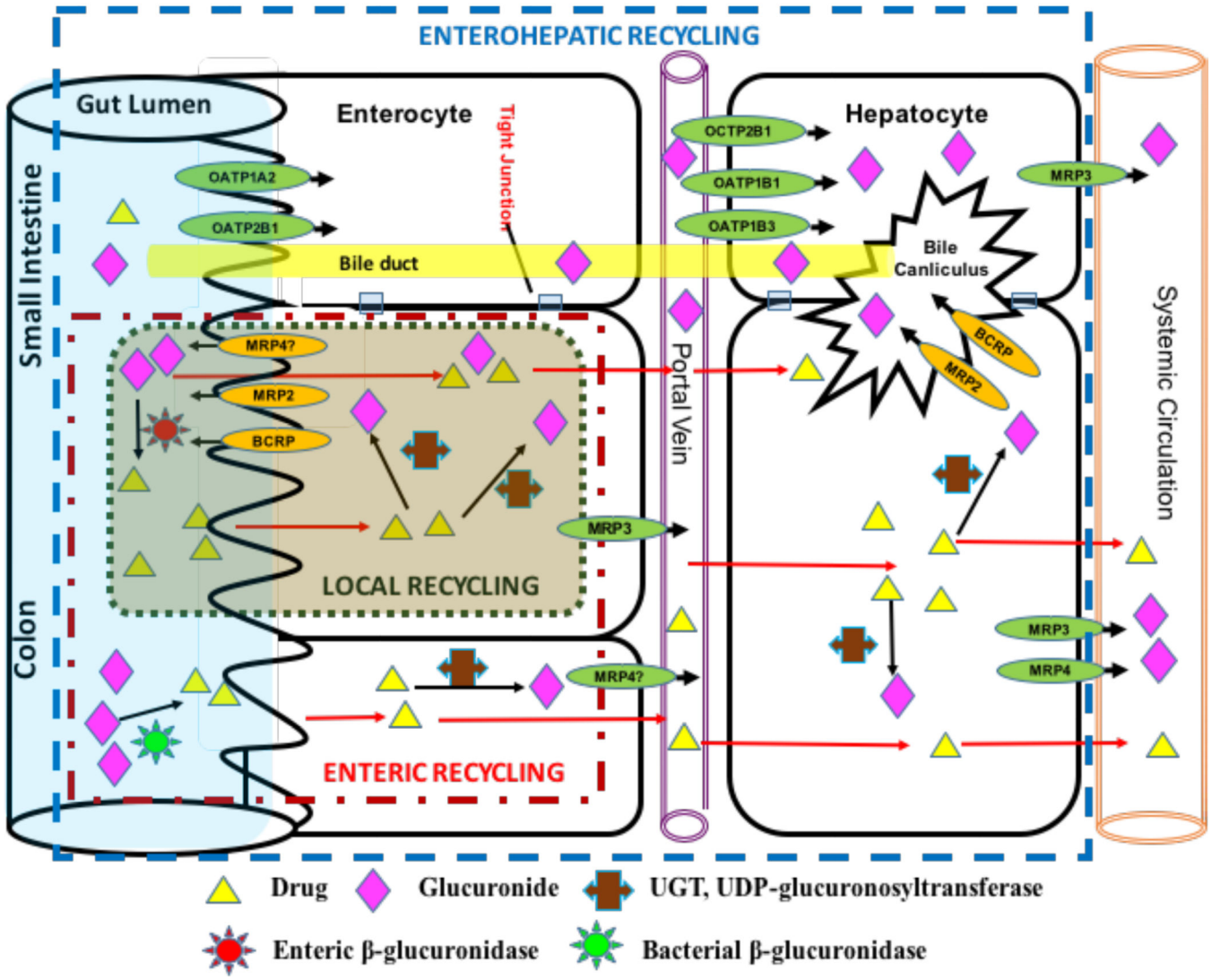



**Figure 2.**

Graphs illustrating mechanisms of enterohepatic recycling, enteric recycling, and local recycling of drug via enteric and hepatic glucuronidation, and bacterial and enteric β-glucuronidases. Enzymatic reaction by UGT and β-glucuronidases from enterocytes/bacteria was marked with black arrow(s), and passive diffusion of drug was marked with red arrow. The local recycling only need the involvement of players enclosed in the dashed green box, whereas enteric and enterohepatic recycling need the involvement of players enclosed in the dashed red and blue boxes, respectively. Within the local recycling, enteric β-glucuronidase is responsible for deconjugation of glucuronide into aglycone, whereas in enteric and enterohepatic recycling mechanisms, bacterial β-glucuronidase is required.

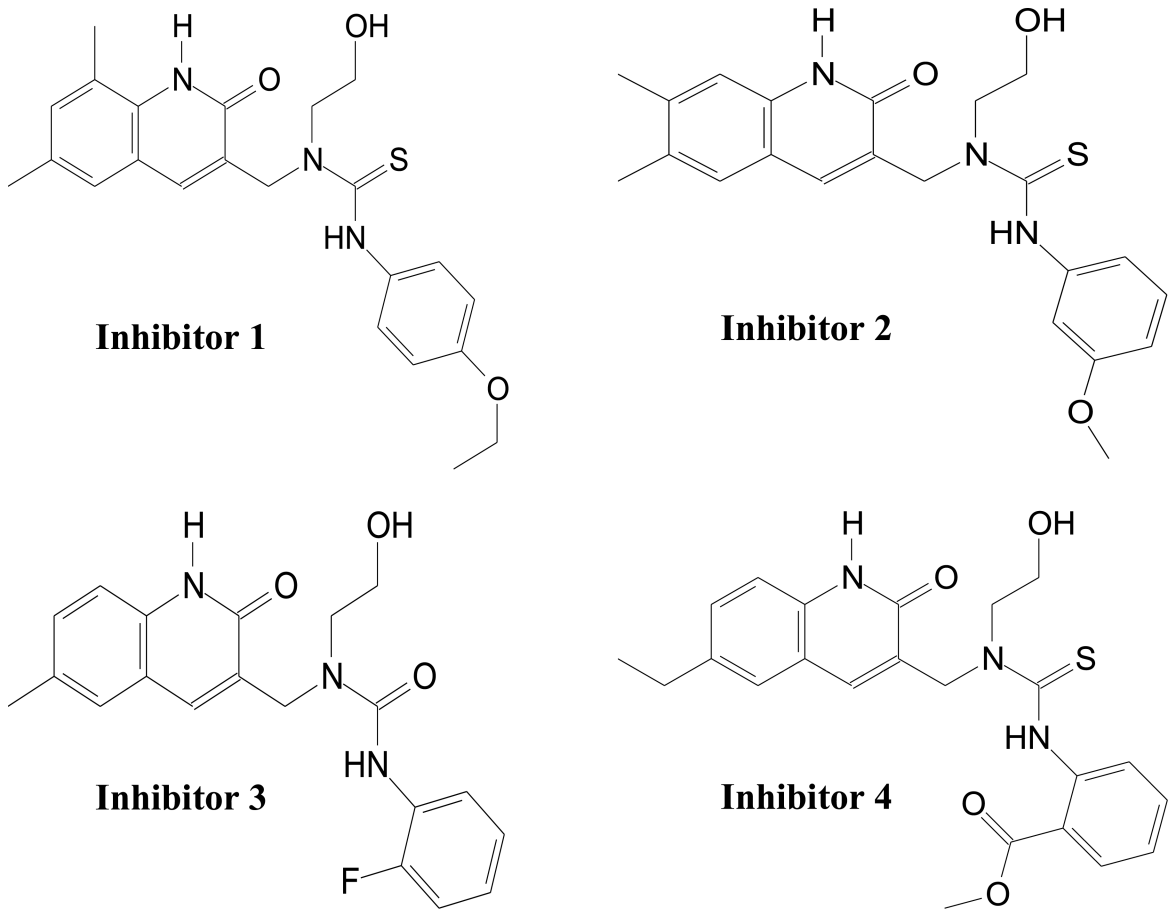
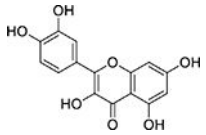
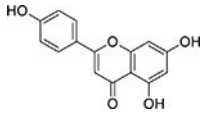
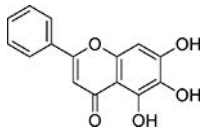
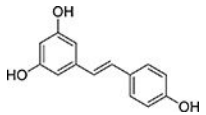
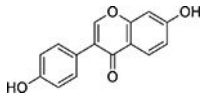
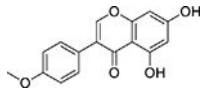
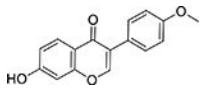
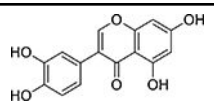
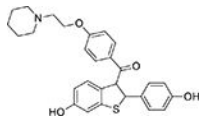
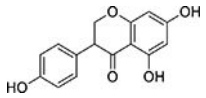
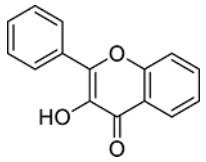
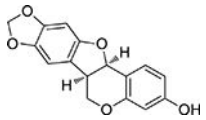
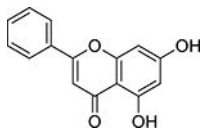
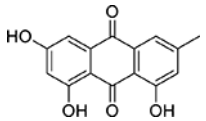
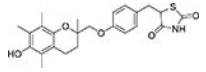


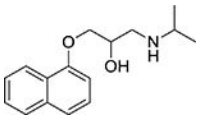
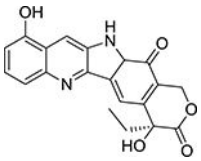
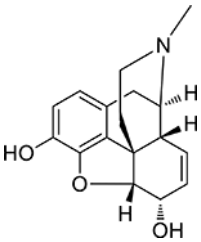
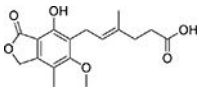
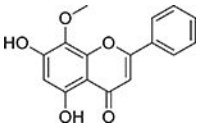
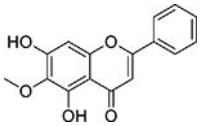

**Figure 3.**
Structures of four selective bacterial β-glucuronidase inhibitors identified via high-throughput screening

**Table 1.**

A list of drugs, endogenous compounds that undergo glucuronidation as their primary clearance mechanism in animals including humans.

| Compound | ACS Number | Chemical Structure | Pathway | Metabolite found in | Species | References |
|---|---|---|---|---|---|---|
| Quercetin | 117-39-5 |  | About 8.5% | Blood, Urine | Rat, mice, gerbil, human | (Manach et al., 1995; Hollman et al., 1997; Morand et al., 1998; Yeh et al., 2016) |
| Apigenin | 520-36-5 |  | Primary, Immature 10.0–31.6% mature 4.9% | Urine, feces, blood | Human, rat, | (Gradolatto et al., 2005; Chen et al., 2007) |
| Baicalein | 491-67-8 |  | Minor, less than 2.9% | Urine, feces, blood | Human, rat, mice, monkey | (Lai et al., 2003b; Tian et al., 2012) |
| Resveratrol | 501-36-0 |  | Primary, greater than 9–16% | Urine, blood, | Human, mice, rat | (Meng et al., 2004; Walle et al., 2004) |
| Daidzein | 486-66-8 |  | Primary, greater than 11% | Urine, feces, blood | Cat, rat, human, mice | (King and Bursill, 1998; Redmon et al., 2016) |
| Biochanin A | 491-80-5 |  | About 30.8% | Blood, urine, fece | Rat, human, mice | (Jia et al., 2004; Moon and Morris, 2007) |
| Formononetin | 485-72-3 |  | Minor, less than 0.18% | Bile | Rat, human, mice | (Jeong et al., 2005a) (Singh et al., 2011b) |

| Compound | ACS Number | Chemical Structure | Pathway | Metabolite found in | Species | References |
|---|---|---|---|---|---|---|
| Luteolin | 491-70-3 |  | Primary, greater than 48.78% | Blood, urine, feces | Rat, human, mice | (Shimoi et al., 1998; Lin et al., 2015) |
| Raloxifene | 84449-90-1 |  | Primary, greater than 11% | Blood, bile, feces, urine | Human, rat, dog | (Kosaka et al., 2011; Trdan et al., 2011) |
| Naringenin | 480-41-1 |  | Primary, greater than 86% | Blood, urine | Rat, human, mice | (Peng et al., 1998; Xu et al., 2009) |
| 3-Hydroxyflavone | 577-85-5 |  | Primary, about 95% | Blood | Mice | (Xu et al., 2013) |
| Maackiain | 19908-48-6 |  | Primary, about 58% | Blood | Mice | (Gao et al., 2011) |
| Chrysin | 480-40-0 |  | Minor, less than 1% | Blood, urine, bile, feces | Humans, rats, mice | (Walle et al., 2001; Ge et al., 2015) |
| Emodin | 518-82-1 |  | About 40% | Blood, urine, feces, bile | Rats, | (Bachmann and Schlatter, 1981; Shia et al., 2010) |
| Troglitazone | 97322-87-7 |  | Minor, less than 5% | Blood, bile, urine, feces | Rats, humans, mice, dogs, cats | (Kawai et al., 1997; Loi et al., 1999; Michels et al., 2000) |

| Compound | ACS Number | Chemical Structure | Pathway | Metabolite found in | Species | References |
|---|---|---|---|---|---|---|
| Propranolol | 525-66-6 |  | About 17% | Bile, urine, blood, feces | Dogs, humans, rats, sheep | (Routledge and Shand, 1979; Marshall et al., 1981; Lo et al., 1984; Walle et al., 1985; Motheova et al., 1986) |
| SN-38 | 130194-92-2 |  | Primary, about 40% | Bile, blood, urine | Rats, humans | (Lokiec et al., 1995; Itoh et al., 2004; Kato et al., 2011) |
| Morphine | 57-27-2 |  | Primary, Greater than 50% | Blood, urine, cerebrospinal, bile | Humans, Rats, Dogs | (Glare and Walsh, 1991; Wolff et al., 1995; Andersen et al., 1997; Christrup, 1997; Andersen et al., 2003) |
| Mycophenolic acid | 24280-93-1 |  | Primary, about 87% | Blood, bile, urine | Humans, | (Joy et al., 2009) |
| Wogonin | 632-85-9 |  | Minor, about 5.9%, 5.7% were metabolites of its sulfates, | Blood, urine | Humans, rats, | (Lai et al., 2003 a) (Talbi et al., 2014) |
| Oroxylin A | |  | - | Blood, urine | Rats, Humans | (Li et al., 1998; Fong et al., 2014) |

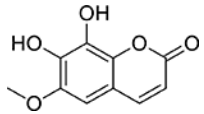
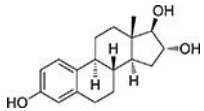
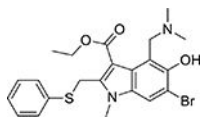
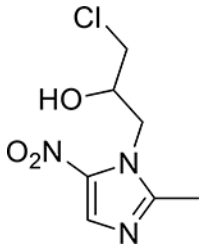
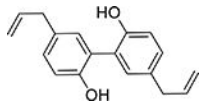
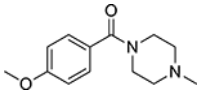
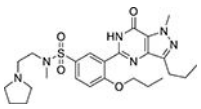
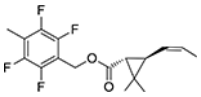
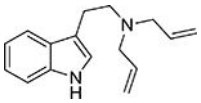
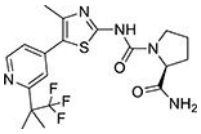
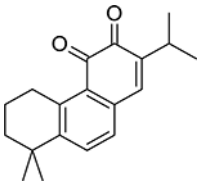
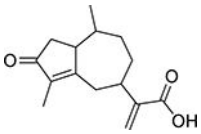
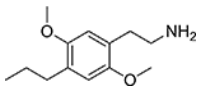
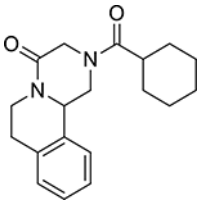
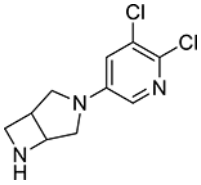
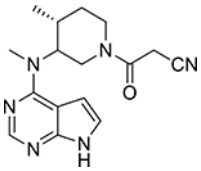
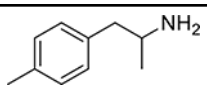
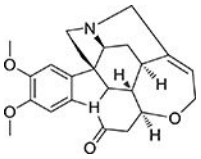
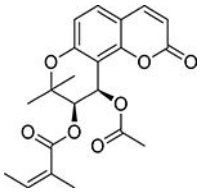
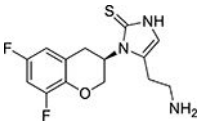
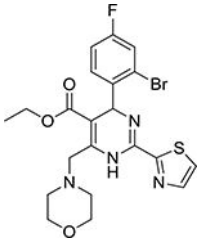
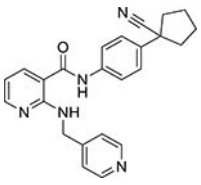
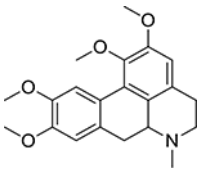
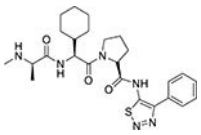
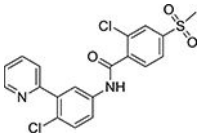
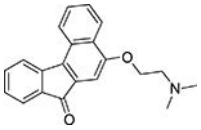
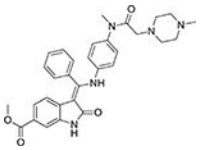
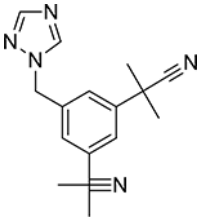
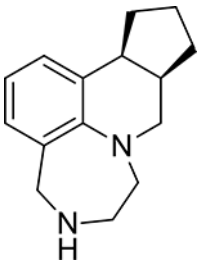
| Compound | ACS Number | Chemical Structure | Pathway | Metabolite found in | Species | References |
|---|---|---|---|---|---|---|
| Fraxetin | 574-84-5 |  | - | Blood | Rats | (Zhao et al., 2016) |
| Estradiol | 50-27-1 |  | Primary, about 53% | Urine, Blood, bile | Humans, Sheep, dogs, baboon, rabbits | (Miyazaki et al., 1972; Musey et al., 1973; Collins et al., 1976; Nambara and Kawarada, 1977; Miyazaki et al., 1980) |
| Arbidol | 131707-25-0 |  | Minor, about 3.6% | Blood, feces, urine | Humans, Rats | (Liu et al., 2012b; Pan et al., 2013) |
| Ornidazole | 16773-42-5 |  | About 37.3% | Blood, urine | Humans, rats, dog | (Schwartz et al., 1979; Du et al., 2013; Du et al., 2014) |
| Magnolol | 528-43-8 |  | Primary, about 18% | Urine, blood, feces, bile | Rats, humans | (Hattori et al., 1986; Nakazawa et al., 2003) |

**Table 2.**

A list of compounds with their secondary metabolic pathway as glucuronidation

| Compounds | ACS Number | Chemical Structures | References |
|---|---|---|---|
| MeOP | 67023-02-3 |  | (Meyer et al., 2015) |
| TPN729 | - |  | (Zhu et al., 2016) |
| Profluthrin | 223419-20-3 |  | (Beyerle et al., 2015) |
| DALT | 60676-77-9 |  | (Michely et al., 2015) |
| Alpelisib | 1217486-61-7 |  | (James et al., 2015) |
| Miltirone | 27210-57-7 |  | (Guo et al., 2015) |
| Rupestonic acid | 115473-63-7 |  | (Gu et al., 2015) |
| 2C-P | 207740-22-5 |  | (Wink et al., 2015) |
| Praziquantel | 55268-74-1 |  | (Wang et al., 2014) |

| Compounds | ACS Number | Chemical Structures | References |
|---|---|---|---|
| ABT-894 | 799279-80-4 |  | (Liu et al., 2014) |
| Tofacitinib | 477600-75-2 |  | (Dowty et al., 2014) |
| 4-MA | 64-11-9 |  | (Welter et al., 2014) |
| Brucine | 357-57-3 |  | (Tian et al., 2014) |
| Praeruptorin A | 73069-27-9 |  | (Song et al., 2014) |
| Etamicastat | 760173-05-5 |  | (Loureiro et al., 2014) |
| GLS4 | - |  | (Zhou et al., 2013) |
| Apatinib | 811803-05-1 |  | (Ding et al., 2013) |

| Compounds | ACS Number | Chemical Structures | References |
|---|---|---|---|
| Glaucine | 475-81-0 |  | (Meyer et al., 2013) |
| GDC-0152 | 873652-48-3 |  | (Yue et al., 2013) |
| Vismodegib | 879085-55-9 |  | (Yue et al., 2011) |
| Benfluron | 78250-23-4 |  | (Jirasko et al., 2011) |
| BIBF 1120 | 928326-83-4 |  | (Stopfer et al., 2011) |
| Anastrozole | 120511-73-1 |  | (Kamdem et al., 2010) |
| Vabicaserin | 620948-93-8 |  | (Tong et al., 2010) |

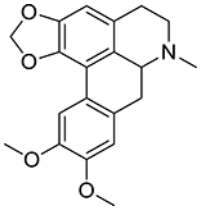
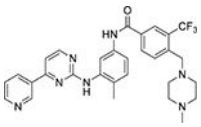
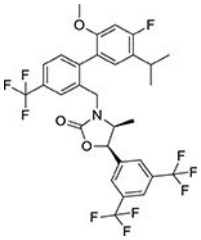
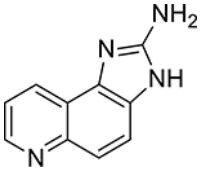
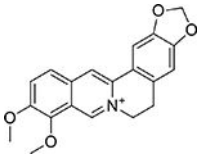
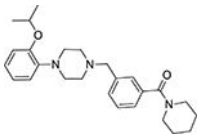
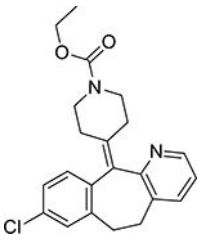
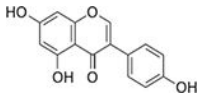
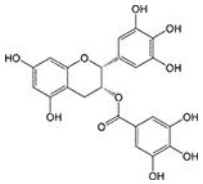
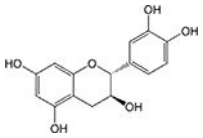
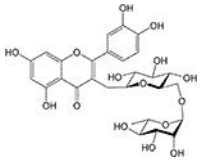
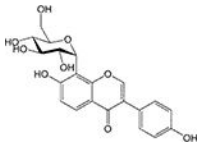
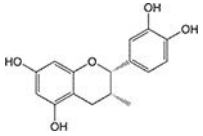
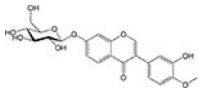
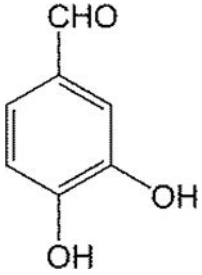
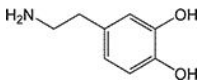
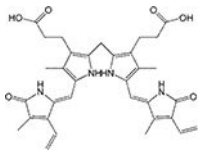
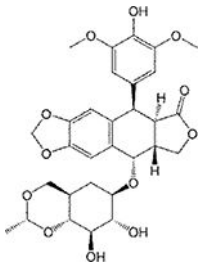
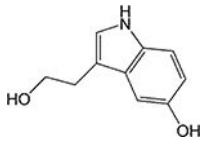
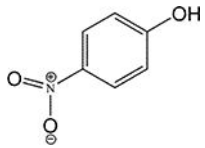
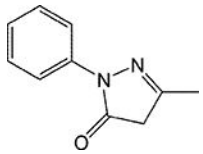
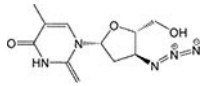
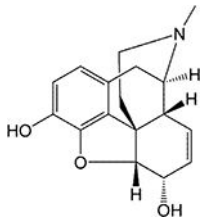
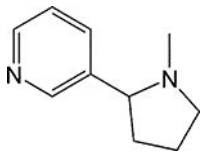
| Compounds | ACS Number | Chemical Structures | References |
|---|---|---|---|
| Dicentrine | 517-66-8 |  | (Lai et al., 2010) |
| Flumatinib | 895519-91-2 |  | (Gong et al., 2010) |
| Anacetrapib | 875446-37-0 |  | (Tan et al., 2010) |
| 2-Amino-3-methylimidazo[4,5-f]quinolone | 76180-96-6 |  | (Lakshmi et al., 2009) |
| Berberine | 2086-83-1 |  | (Liu et al., 2009) |
| Mazapertine | 134208-17-6 |  | (Wu et al., 2007) |
| Loratadine | 79794-75-5 |  | (Ramanathan et al., 2007) |

**Table 3.**

Glucuronidation of highly water soluble compounds

| Compound | ACS Number | Chemical Structure | Glucuronidation Pathway | Metabolite found in | Species studied | References |
|---|---|---|---|---|---|---|
| Genistein | 446-72-0 |  | Primary, greater than 50% | Urine, feces and blood | Mice, rats, humans | (Adlercreutz et al., 1993; Bloedon et al., 2002; Busby et al., 2002; Chen et al., 2003a; An and Morris, 2011; Yang et al., 2012) |
| EGCG | 989-51-5 |  | Minor, about 7.5% | Blood, urine, bile, | Rats, mice, humans, dogs | (Vaidyanathan and Walle, 2002) |
| Catechin | 88191-48-4 |  | Primary, greater than 50% | Blood, bile, urine, | Rats, mice, dogs, monkeys, humans | (Kuhnle et al., 2000) |
| Rutin | 153-18-4 |  | Rarely, about 2%. Most were metabolites of its aglycone | Blood, urine, | Rats, humans, filamentous fungi | (Jaganath et al., 2006) |
| Puerarin | 3681-99-0 |  | Minor, about 5%. Some were metabolites of its aglycone | Blood, urine, bile | Rats, humans | (Luo et al., 2012) |
| Epicatechin | 35323-91-2 |  | Primary, greater than 45% | Blood, | Rats, humans, dogs, | (Crespy et al., 2004) |

| Compound | ACS Number | Chemical Structure | Glucuronidation Pathway | Metabolite found in | Species studied | References |
|---|---|---|---|---|---|---|
| Calycosin-7-O-Glucuronide | 20633-67-4 |  | Minor, about 5% | Blood, | Rats | (Shi et al., 2015) |
| Protocatechuic Aldehyde | 139-85-5 |  | greater than 30% | Urine | Rats, pigs | (Liu et al., 2008; Zhao et al., 2013) |
| Dopamine | 51-61-6 |  | greater than 25% | Brain | Rats, mice | (Uutela et al., 2009) |
| Bilirubin | 635-65-4 |  | greater than 50% | Liver | Human | (Ma et al., 2014) |
| Etoposide | 33419-42-0 |  | greater than 50% | Blood, urine, bile | Rats, rabbits, humans | (Hande et al., 1988a; Hande et al., 1988b; Wen et al., 2007) |

| Compound | ACS Number | Chemical Structure | Glucuronidation Pathway | Metabolite found in | Species studied | References |
|---|---|---|---|---|---|---|
| 5-Hydroxytryptophol | 154-02-9 |  | greater than 50% | Urine, | Humans, | (Stephanson et al., 2005) |
| 4-Nitrophenol | 100-02-7 |  | About 40% | Urine, bile | Rats, | (Meerman et al., 1987) |
| Edaravone | 89-25-8 |  | greater than 70% | Urine | humans | (Ma et al., 2012) |
| Zidovudine | 30516-87-1 |  | greater than 50% | Blood, | Humans, | (Nadal et al., 1996) |
| Morphine | 57-27-2 |  | greater than 50% | Blood, muscle, fat, brain | Humans, | (Frost et al., 2015) |
| Nicotine | 22083-74-5 |  | Minor, about 5–10% | Blood, | Humans, | (Kaivosaari et al., 2007) |

| Compound | ACS Number | Chemical Structure | Glucuronidation Pathway | Metabolite found in | Species studied | References |
|---|---|---|---|---|---|---|
| Imipramine | 50-49-7 |  | greater than 20% | Blood, urine | Humans, rabbits | (Qian and Zeng, 2006) |
| Benzotriazole | 95-14-7 |  | greater than 20% | Blood | Rats, | (Town et al., 1993) |
| Chloramphenicol | 56-75-7 |  | greater than 50% | Liver | bovine, equine, porcine | (Fedeniuk et al., 2015) |