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# Interaction of soy isoflavones and their main metabolites with hOATP2B1 transporter

Lucie Navrátilová[1] · Lenka Applová[1] · Pavel Horký[2] · Přemysl Mladěnka[1] · Petr Pávek[1] · František Trejtnar[1]

## Abstract

Membrane organic anion-transporting polypeptides (OATPs) are responsible for the drug transmembrane transport within the human body. The function of OATP2B1 transporter can be inhibited by various natural compounds. Despite increased research interest in soya as a part of human diet, the effect of its active components to interact with hOATP2B1 has not been elucidated in a complex extent. This in vitro study examined the inhibitory effect of main soy isoflavones (daidzin, daidzein, genistin, genistein, glycitin, glycitein, biochanin A, formononetin) and their metabolites formed in vivo (S-equol, O-desmethylangolensin) towards human OATP2B1 transporter. MDCKII cells overexpressing hOATP2B1 were employed to determine quantitative inhibitory parameters of the tested compounds and to analyze mechanism/s of the inhibitory interaction. The study showed that aglycones of soy isoflavones and the main biologically active metabolite S-equol were able to significantly inhibit hOATP2B1-mediated transport. The $K_i$ values for most of aglycones range from 1 to 20 μM. In contrast, glucosides did not exhibit significant inhibitory effect. The kinetic analysis did not indicate a uniform type of inhibition towards the hOATP2B1 although predominant mechanism of inhibition seemed to be competitive. These findings may suggest that tested soy isoflavones and their metabolites might affect transport of xenobiotics including drugs across tissue barriers via hOATP2B1.

**Keywords** Soy isoflavones · OATP · Drug transporter · SLCO · Flavonoids

## Abbreviations

| | |
|---|---|
| BCA | Bicinchoninic acid |
| BSA | Bovine serum albumin |
| DMSO | Dimethyl sulfoxide |
| E3S | Estrone 3-sulfate |
| MDCKII | Madin-Darby canine kidney II |
| OATP | Organic anion transporting polypeptide |
| ODMA | O-Desmethylangolensin |
| SLC | Solute carrier |

✉ František Trejtnar
trejtnarf@faf.cuni.cz

1 Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

2 Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

## Introduction

Asian population has been consuming foods from soya for centuries, whereas in the rest of the world, the increase occurred in the last decade of the twentieth century (Wang et al. 2013). Soya and soy proteins (such as tofu, soy milk, or soy yoghurt) are nowadays common food; they are also contained in bakery products, meat products, or infant formulas (Ritchie et al. 2006; Grosser et al. 2015). New diet trend to minimize intake of a meat and become vegetarian or vegan leads to the further increase of soyfood consumption. In addition, soy components are obtained in dietary supplements recommended for prevention or treatment of various pathologies (Delmonte and Rader 2006). Soya is rich in isoflavones, which were demonstrated to possess numerous positive biological actions, e.g., phytoestrogenic effect (with consequent antiosteoporotic effect and reduction of menopausal symptoms), antioxidant effect, inhibitory effect on the proliferation of cancer cells, and anti-inflammatory effect (Wang et al. 2013; Grosser et al. 2015). The phytoestrogenic effects are based mainly on the structural similarity of isoflavones to steroidal estrogens and the binding to estrogen receptors (Franke et al. 2014).

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The soy isoflavones can be found in different forms: glucosides daidzin, genistin, glycitin or aglycones daidzein, genistein, glycitein, biochanin A, and formononetin (Nakamura et al. 2000; Mattison et al. 2014). Formononetin and biochanin A, precursors to daidzein and genistein, respectively, occur also in red clover, sprouts, chick peas, black bean seeds, etc. (Larkin et al. 2008; Harini et al. 2012). In the small intestine, glucosides are hydrolyzed to their appropriate aglycones. In addition, they can be further metabolized by the intestinal microflora to other metabolites (Larkin et al. 2008; Mattison et al. 2014). Daidzein can be metabolized in the intestine by reduction to *S*-equol or by ring cleavage to *O*-desmethylangolensin (ODMA) and genistein to 6′-hydroxy-*O*-desmethylangolensin (Fig. 1) (Larkin et al. 2008). *S*-Equol is proposed to play a major role in the health benefits of soy products (Mattison et al. 2014; Franke et al. 2014). For example, it has been tested in clinical studies for the treatment of menopausal symptoms (Jenks et al. 2012; Thomas et al. 2014). Also, animals commonly produce *S*-equol and *O*-desmethylangolensin after ingestion of isoflavones. Therefore, animal products, especially milk and milk products, may be a secondary source of the isoflavone metabolites for human population (Mattison et al. 2014; Kašparovská et al. 2017).

Many studies have documented that flavonoid components contained in food or herbal preparations are modulators of drug metabolizing enzymes and/or drug transporters (Meng and Liu 2014; Li et al. 2017). Experimental evidence suggest that the interactions of natural compounds with these systems may change pharmacokinetics of drugs significantly (Berginc et al. 2010; Choi et al. 2010; Meng and Liu 2014). Dietary isoflavones may inhibit activity both the efflux ABC transporters as well as uptake transporters such as organic anion transporters (OATs), organic cation transporters (OCTs), and/or organic anion-transporting polypeptides (OATPs) (Bircsak and Aleksunes 2015; Taneja et al. 2016; Li et al. 2017). Because OATPs play an important role in a transport of endo- as well as xenobiotics across plasma membranes, they can affect transport processes in organs critical for the drug disposition such as the liver, intestine, kidney, placenta, and blood-brain barrier (Hagenbuch and Stieger 2013). These transporters mediate the sodium-independent transport of a wide spectrum of organic anions and amphipathic compounds, including numerous drugs and other xenobiotics (Hagenbuch and Stieger 2013; Estudante et al. 2013). Their substrates include members of clinically important drug groups, such as statins, antibiotics, beta-blockers, antihistamines, antidiabetics, or anticancer drugs (Shitara et al. 2013; Kovacsics et al. 2017).

The hOATP2B1 transporter is expressed in the organism in several barriers determining pharmacokinetics of drugs. A significant hOATP2B1 expression has been proved at least in the liver, intestine, placenta, and blood-brain barrier

(Hagenbuch and Stieger 2013; Kovacsics et al. 2017). The expression of hOATP2B1 in human hepatocytes is lower than hOATP1B1 but comparable to hOATP1B3 (Badée et al. 2015; Vildhede et al. 2018). All the transporters have been found to be localized in the basolateral membrane of hepatocytes (Kullak-Ublick et al. 2001) where they are responsible for drug uptake from the blood into the liver (Hagenbuch and Stieger 2013). The hOATP2B1 also exhibits a significant expression in all segments along the human intestine (Meier et al. 2007; Drozdzik et al. 2014). Despite its relative significant amount in the intestine, its meaning for transport of drugs is difficult to assess due to inconsistent data on its exact cellular localization (Kobayashi et al. 2003; Keiser et al. 2017).

A potential inhibition of important drug transporters by natural compounds such as soy isoflavones could be considered as a potential source of drug-food interactions. The multiple organ expression of hOATP2B1 could suggest that its potential inhibition by diet isoflavones might affect drug uptake in pharmacodynamically relevant tissues. Therefore, the aim of the presented in vitro study was to assess the interactions of soy isoflavones and their selected metabolites with the human OATP2B1 transporter and determine kinetic parameters of these interactions.

## Experimental section

### Chemicals

Daidzein (≥ 98% purity), genistein (≥ 98%), and estrone 3-sulfate sodium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA); formononetin (≥ 99%) was purchased from Phytolab (Vestenbergsgreuth, Germany); *S*-equol (97%) was purchased from Toronto Research Chemicals (Toronto, Canada); biochanin A (≥ 99%), daidzin (≥ 99%), genistin (≥ 99%), glycitein (≥ 95%), and glycitin (≥95%) were purchased from Extrasynthese (Lyon, France). *O*-Desmethylangolensin (ODMA) was synthetized at the Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Charles University, Czech Republic. For the experiments, the compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich). [$^3$H]-Estrone 3-sulfate (E3S, 50 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA).

### Cell culture

Madin-Darby canine kidney II (MDCKII) cell line was purchased from the European Collection of Cell Culture (Salisbury, UK). The MDCKII cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich). The MDCKII cells were subcultured by
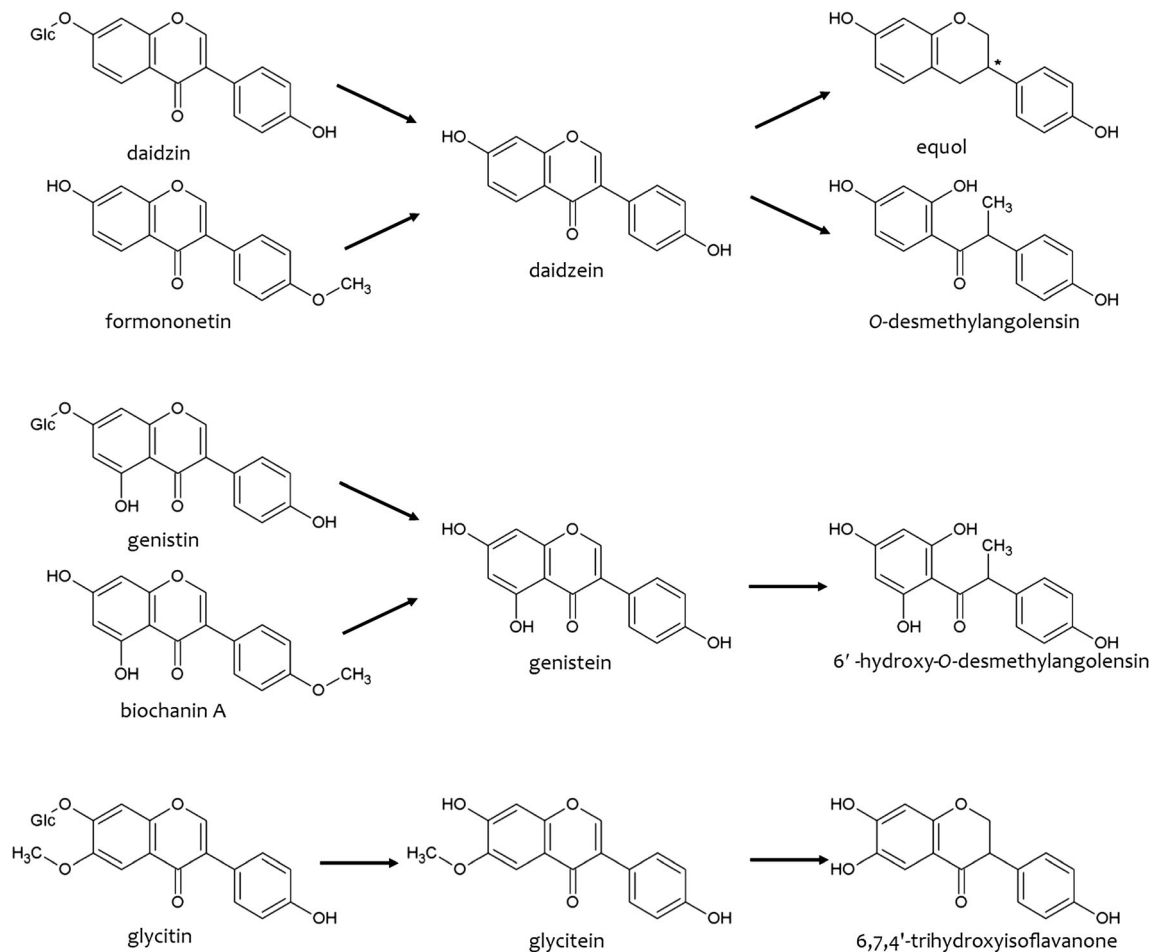
**Fig. 1** The proposed intestinal metabolic pathway of soy isoflavones (Joannou et al. 1995; Heinonen et al. 2003; Larkin et al. 2008)

trypsinization using 0.25% trypsin/EDTA solution (Sigma-Aldrich). The cells were maintained in a humidified atmosphere containing 5% $CO_2$ at 37 °C.

## Transient transfection

The MDCKII cells were seeded at a density of $10^5$ cells per well in 24-well plates. Plasmid hOATP2B1 (0.6 μg/well) was transfected into MDCKII cells 24 h after seeding (confluence of ~95%) using Lipofectamine 2000 (1.2 μl/well) (Invitrogen, Carlsbad, CA, USA). The cells transfected with empty vector (pCMV6-Entry vector) under the same transfection conditions served as a control (mock cells). Both plasmids were obtained from OriGene Technologies (Rockville, MD, USA). This cellular model in more details is described in the previous publication of Navrátilová et al. (2017). The overexpression of the studied transporter was confirmed by Western blot analysis (Navrátilová et al. 2017) and by function transport tests using an accumulation study with radiolabeled standard substrate [3H]-estrone 3-sulfate (E3S) in each experiment.

## Inhibitory transport assays

Inhibitory transport assays were carried out in 24-well plates. Transport analysis in MDCKII cells transiently transfected with hOATP2B1 was performed 24 h after transfection. Following the removal of the cultivation medium, the cells were washed with the prewarmed (37 °C) transport solution and preincubated for 10 min at 37 °C. The transport solution contained 130 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2·6H_2O$, 5 mM glucose, and 10 mM HEPES, pH 7.4. An initial comparative study on inhibitory potency of isoflavones and their metabolites towards hOATP2B1 determined uptake of standard radiolabeled substrate [3H]-estrone 3-sulfate (1 μM) into MDCKII-OATP2B1 cells in absence (control) or presence of the tested compound at the concentration of 100 μM. For kinetic studies, the hOATP2B1-mediated uptake of estrone 3-sulfate at three different concentrations was measured in the absence and presence of increasing concentrations of studied isoflavones (0–250 μM) with maximal concentration of DMSO 0.5%. The substrate E3S was a mix of radiolabeled (20 nM) and non-radiolabeled compound to reach desired concentrations.

The substrate and the isoflavones dissolved in the transport solution were added to the cell monolayers and incubated for 2 min. After this time period, incubation was terminated by washing the cells three times with 1 mL ice-cold solution containing 137 mM NaCl and 10 mM HEPES, pH 7.4. The cells were lysed with 0.2 mL of Triton X 0.5% in 100 mM NaOH for 20 min at 37 °C. The intracellular uptake of the radioactivity was determined by liquid scintillation counting (Tri-Carb 2900TR, Perkin Elmer, Shelton, USA). All the values were standardized against protein content, which was determined using the BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA) with reference to BSA as standard.

The experiments were performed in triplicates with minimally two separate measurements.

## Data and statistical analysis

The percentage of E3S uptake inhibition by isoflavones was related to the control with the total absence of isoflavones (100% uptake). Results were expressed as the concentration of inhibitor $IC_{50}$, which resulted in the half-rate inhibition of the substrate uptake for each substrate concentration. Inhibitory constant ($K_i$) and the type of an inhibition were determined from Dixon plots (reciprocal velocity is plotted against the concentration of inhibitor) and Lineweaver-Burk graphs, respectively, using GraphPad Prism software (version 7). Data are presented as mean ± SD. Statistical differences were evaluated by $t$ test. A value of $p < 0.05$ was considered significant.

## Results

### Comparison of inhibitory potency

To investigate whether hOATP2B1-mediated uptake is inhibited by the tested soy isoflavones or metabolites, the uptake of estrone 3-sulfate (E3S) into MDCKII-OATP2B1 was firstly examined in the absence or presence of these compounds at their concentration 100 μM. As shown in Fig. 2, comparison of E3S uptake did not prove any significant inhibitory effect towards hOATP2B1 transporter for glucosides daidzin, genistin, and glycitin at the used concentration. On the contrary, all of the tested aglycones and metabolites inhibited hOATP2B1-mediated cell uptake significantly at the tested concentration (Fig. 2).

### Quantitative inhibitory parameters

All soy isoflavones with a significant inhibitory effect on hOATP2B1 uptake were further studied to determine the quantitative inhibitory parameters and the type of inhibition. The concentration-dependent inhibitory effect was observed for all of the tested compounds (Fig. 3). Except for
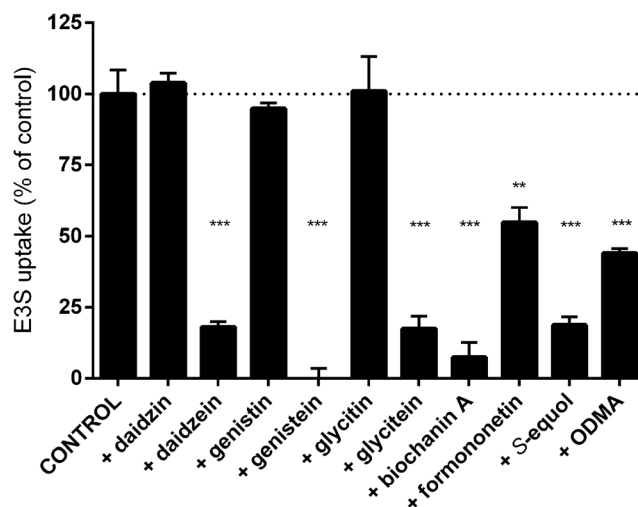


Fig. 2 The effect of isoflavones on the hOATP2B1-mediated E3S uptake. The E3S (1 μM) uptake was measured in absence (control) or presence of soy isoflavones and metabolites (100 μM). Cells were incubated with isoflavones for 2 min at 37 °C. The inhibition of the uptake is expressed as the percentage of control. Data are expressed as mean ± SD ($n = 9$). *** $p < 0.001$, ** $p < 0.01$ significantly different from control

formononetin, all soy aglycones showed relatively strong inhibitory potency towards hOATP2B1 with $K_i$ values ranging from 1 to 20 μM (Fig. 4) with the most potent inhibitors biochanin A with $K_i = 1.4 \pm 1.7$ μM (Fig. 4d) and genistein with $K_i = 4.6 \pm 2.5$ μM (Fig. 4b).

Our results document a relatively strong inhibitory potency of S-equol towards hOATP2B1 uptake activity with $K_i$ values $18.6 \pm 5.6$ μM (Fig. 5a). On the other hand, the metabolite ODMA exhibited a low inhibitory potency with $K_i$ almost an order of magnitude higher (Fig. 5b).

### Mechanisms of inhibition

The kinetic analyses using E3S as a hOATP2B1 standard substrate indicated that most of the tested isoflavones and both metabolites inhibited this transporter competitively (Figs. 4 and 5). However, genistein and biochanin A showed noncompetitive way of inhibition of the hOATP2B1 (Fig. 4b, d).

## Discussion

The presented results showed a significant inhibitory potency of several soy isoflavones and their metabolites towards OATP2B1 transporter. Such finding is in a good agreement with the previously published results on the inhibition of OATP2B1-mediated transport observed in soy extract (Fuchikami et al. 2006). The performed study shows that OATP2B1 inhibition by soy products is at least partly dependent on isoflavone inhibition activity. The obtained data
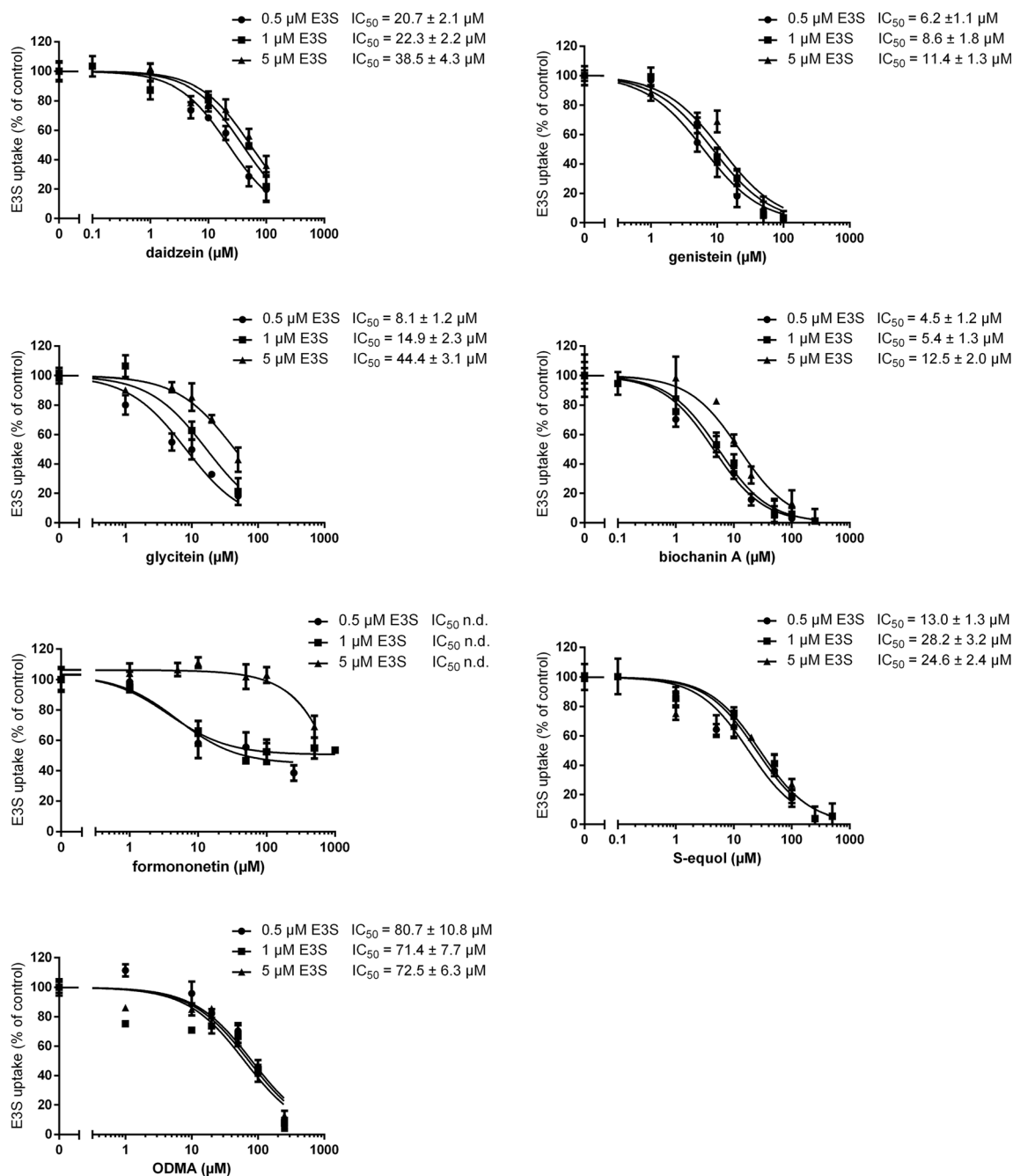
**Fig. 3** The inhibition of the hOATP2B1-mediated E3S uptake by isoflavones and their metabolites. The concentration-dependent inhibitory effect of daidzein, genistein, glycitein, biochanin A, formononetin, S-equol, and ODMA on the uptake of E3S in three different concentrations of this substrate (0.5, 1, and 5 μM) into transiently transfected MDCKII-OATP2B1 cells was measured after 2 min of incubation at 37 °C in triplicates. The concentration of isoflavonoids was 0–500 μM (for formononetin 0-1000 μM). The values of the uptake of E3S into the mock cells were subtracted. Each point represents the mean ± SD ($n = 6$–8) (n.d. not determined). These data were used for $K_i$ determination and plots

suggest which soy isoflavones may be responsible for the inhibitory effect towards OATP2B1 transporter. A clear difference in inhibitory potency towards hOATP2B1 in conjugated isoflavones and their appropriate aglycones was observed. With one exclusion, the aglycones inhibited the transporter with $K_i$ values ranging from 1 to 20 μM (Figs. 4 and 5).

On the other hand, the tested glucosides daidzin, genistin, and glycitin did not exhibit a significant potency to inhibit hOATP2B1 at the used concentration range (Fig. 2). The results in the pair genistin/genistein demonstrated that only the aglycone had an effect on hOATP2B1 transporter. This finding is in accordance with an observation for the related

**Fig. 4** The inhibition of the hOATP2B1-mediated E3S uptake by isoflavone aglycones. Dixon plots of the inhibition of E3S uptake (left column) at three different concentrations of E3S (black circle 0.5 μM; black square 1 μM; black up-pointing triangle 5 μM E3S). Uptake was determined in the absence or presence of increasing concentration of isoflavone aglycones after 2 min incubation at 37 °C. The reciprocal velocity is plotted against the concentration of inhibitor. Lineweaver-Burk plots (right column) were determined to illustrate the type of inhibition (black circle absence of inhibitor, black square presence of inhibitor in the 100 μM concentration). ($n = 6$–8)
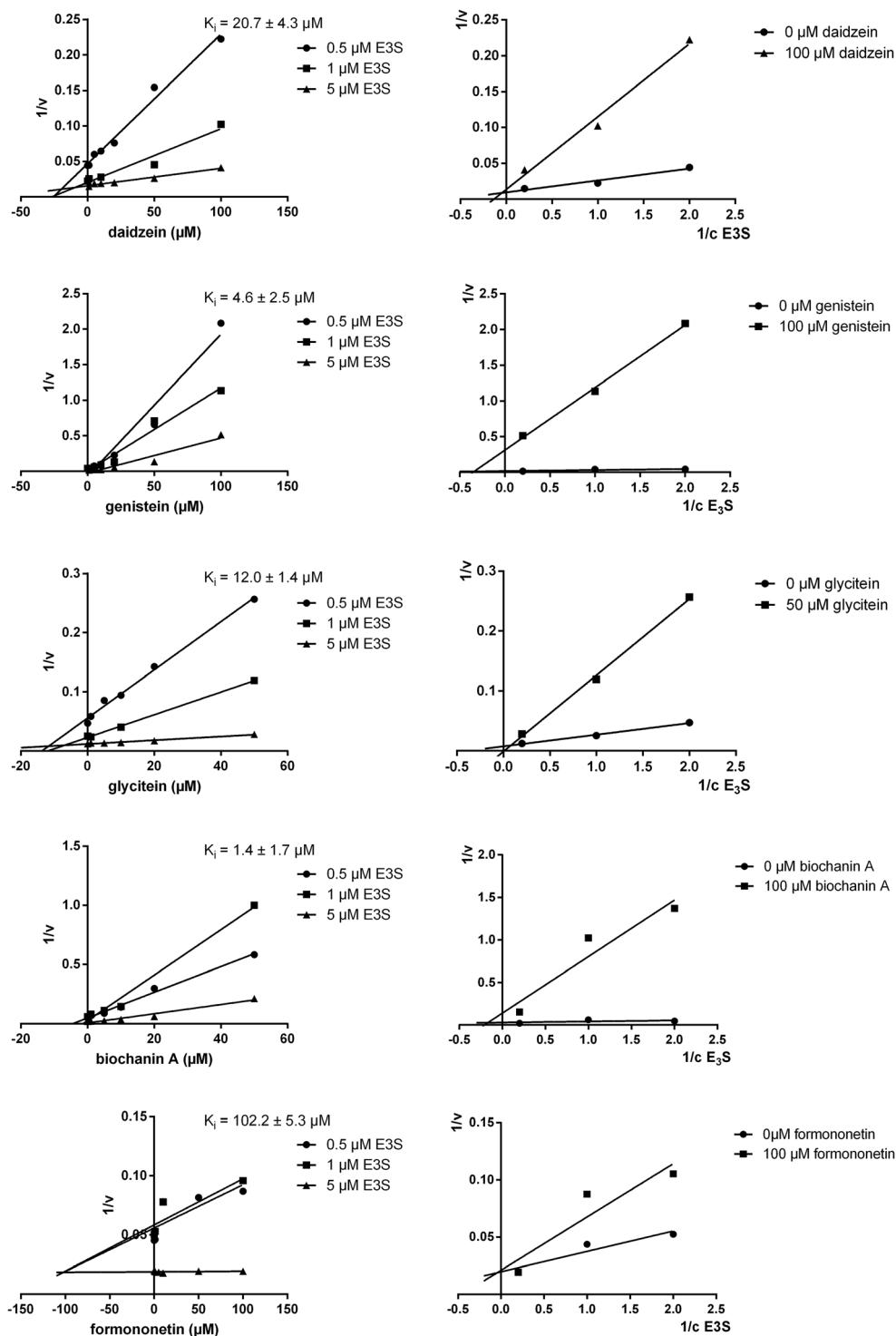


transporter hOATP1B1 which was inhibited only by the aglycone genistein (Wang et al. 2005). Similar difference in the inhibitory potency towards hOATP2B1 was observed also for flavonoid compounds, such as the pair diosmin/diosmetin (Wang et al. 2005). On the other hand, some flavonoid glycosides exhibited the ability to inhibit hOATP1B1 similarly as their appropriate aglycones. Such finding was observed for couples hesperidin/hesperitin, naringin/naringenin, or phloridzin/phloretin (Wang et al. 2005).

Several other groups of flavonoid compounds have been proved to inhibit OATP2B1 transporter. Among the potent inhibitors belong active components of grapefruit and orange

**Fig. 5** The inhibition of the hOATP2B1-mediated E3S uptake by metabolites of isoflavones. Dixon plots of the inhibition of E3S uptake (left column) at three different concentrations of E3S (black circle 0.5 μM; black square 1 μM; black up-pointing triangle 5 μM E3S). Uptake was determined in the absence or presence of increasing concentration of isoflavone metabolites after 2 min incubation at 37 °C. The reciprocal velocity is plotted against the concentration of inhibitor. Lineweaver-Burk plots (right column) were determined to illustrate the type of inhibition (black circle absence of inhibitor, black square presence of inhibitor in the 100 μM concentration). ($n = 6$–8)
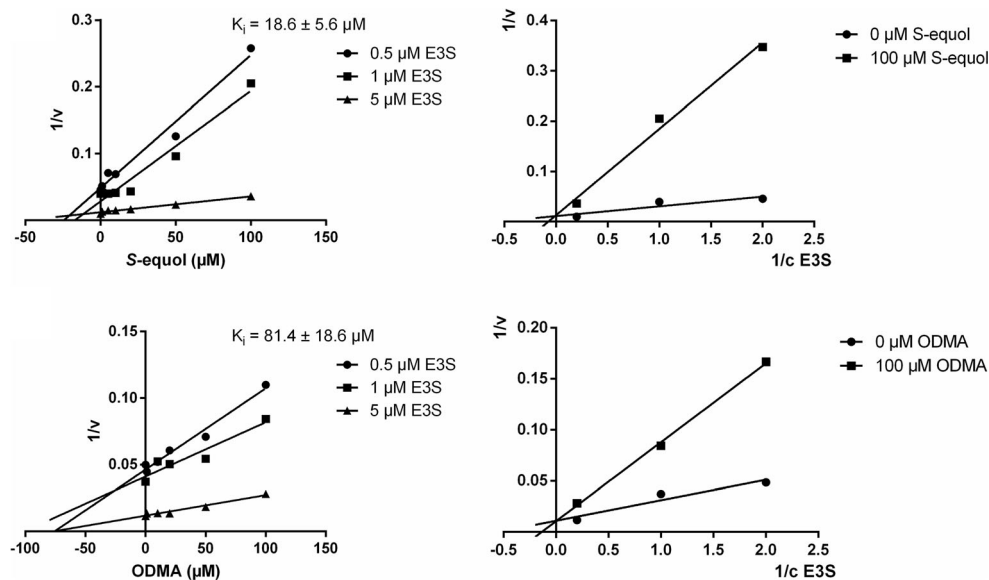


juices, such as naringin and hesperidin (Shirasaka et al. 2013). Flavonoids quercetin, kaempferol, apigenin, or flavonolignan silymarin were also demonstrated to be other potent herbal OATP2B1 inhibitors (Mandery et al. 2010; Köck et al. 2013). The determined inhibitory concentrations found in the soy isoflavones seem to be comparable with those found in the other flavonoids, such as quercetin, kaempferol, and apigenin, but such comparison is difficult due to differences in the used standard OATP2B1 substrates.

The found inhibition effect of the soy isoflavones on the OATP2B1-mediated transport and some previous reports may suggest that the inhibitory ability towards human OATPs could be a general characteristic of selected soy isoflavones. Despite scarce experimental data, soy isoflavones seem to be also inhibitors of the specific hepatic transporter hOATP1B1 (Wang et al. 2005). According to the value of $K_i$ found in the study of hOATP1B1 (10.2 μM), biochanin A seems to be stronger inhibitor of OATP2B1 ($K_i = 1.4$ μM) than OATP1B1. However, further studies on interactions of the main soy isoflavones and the other important OATPs are needed to complete our knowledge in this area.

Soy isoflavones are found in the food or food supplements predominantly as glucosides. The aglycones represent only a minor part of the total content of isoflavones in food sources (Song et al. 1998; Nakamura et al. 2000). But the isoflavone glucosides are cleaved in the intestine by glycosidases localized in the upper parts of the gut including the duodenum (Walsh et al. 2007; Larkin et al. 2008; Mattison et al. 2014; Franke et al. 2014). The formed aglycones are then efficiently absorbed into the portal vein (Yuan et al. 2007; Mattison et al. 2014) and then reach the liver where they can affect hepatic transporter systems including OATPs. The potential risk of isoflavone-drug interactions due to OATP2B1 inhibition may be in vivo limited by

the fact that soy isoflavones are further metabolized to glucuronides and sulfates (Mattison et al. 2014) with likely lower biological activity. On the other hand, a complex mixture of the isoflavones found in the most soy products might result in a synergic inhibition of the hOATP2B1. Such effect towards hOATP2B1-mediated uptake was observed in vitro by Shirasaka et al. (2013) for a mixture of flavonoids contained in fruit juices. In addition, a combination with other strong herbal OATP2B1 inhibitors originating from other food sources can intensify the potential inhibitory effect.

Soy isoflavones from the diet are converted in the human GIT not only to appropriate aglycones but also to other metabolites with biological activity, such as S-equol (Yuan et al. 2007). Another potential source of S-equol in the human diet may be bovine milk and milk products (Kašparovská et al. 2017). Due to its considerable hormonal activity, this metabolite is potential agent for relieve of menopausal flashes (Thomas et al. 2014). Because S-equol is readily absorbed to the systemic circulation (Mattison et al. 2014) and exhibits significant inhibitory potential in vitro, it could be considered to be a potential inhibitor of OATP2B1 in vivo, but there are very limited data on its plasma levels following hormonally active doses (Setchell et al. 2009). The fate of the second tested intestinal metabolite ODMA in vivo is not fully known, but its low inhibitory potency in vitro suggests its low potency for systemic food-drug interactions involving hOATP2B1 even in case of a high absorption rate from the intestine.

The mechanism of inhibition by the tested isoflavones towards the hOATP2B1 transporter was not uniform. Most of the tested isoflavones and both metabolites inhibited the hOATP2B1 transporter in a competitive manner (Figs. 4 and 5). Similarly to the tested isoflavones, several other phytochemicals like flavonoids apigenin, kaempferol, and quercetin

were proved to be also the competitive inhibitors of hOATP2B1 and hOATP1A2 transporters (Mandery et al. 2010). Their inhibition of the related liver hOATP1B1 and hOATP1B3 transporters was demonstrated to be competitive too (Mandery et al. 2012). The observed non-competitive type of inhibition by biochanin A is in line with the fact that this isoflavone was previously found to be non-competitive inhibitor of hOATP1B1 (Wang et al. 2005).

OATP2B1 was found to be expressed in several organs and tissues (Roth et al. 2012; Hagenbuch and Stieger 2013). However, the most important role of the hOATP2B1 in drug disposition is likely to be expected in the liver because of the considerable hepatic expression (Badée et al. 2015; Vildhede et al. 2018) and decisive capacity of the liver to affect pharmacokinetics. Similarly, to the typical hepatic OATPs, OATP1B1, and OATP1B3, OATP2B1 may contribute to hepatic clearance of drugs (Koenen et al. 2011; Kovacsics et al. 2017). The hOATP2B1 can be especially significant in the liver uptake of its specific drug substrates. There are several clinically important drugs assumed to be specifically or preferentially transported by hOATP2B1 (e.g., glibenclamide, aliskiren, amiodarone, gefitinib) (Koenen et al. 2011; Roth et al. 2012; Sato et al. 2018). Such substrates with predominant or specific hepatic transport by hOATP2B1 may be potential candidates for transport interactions with concomitantly administered plant OATP2B1 inhibitors such as the tested soy isoflavones.

## Conclusions

The presented in vitro results demonstrate that the tested isoflavones exhibit the significant inhibitory potency towards drug transporting SLC transporter hOATP2B1. The performed head-to-head comparison enabled to identify which isoflavone components of soya could be the main contributors to the inhibitory effect. A significant difference in the inhibitory potency towards hOATP2B1 between glucosides and aglycones was found. The study also proved that not only parent soy isoflavones but also their metabolites formed in vivo are able to inhibit transport via hOATP2B1 transporter. The performed study may contribute to reveal the potential of the soy isoflavones for interactions with the hOATP2B1 substrates including drugs. Therefore, the elucidation of the effect of soy isoflavones on hOATP2B1-mediated transport seems to be an important issue.

## Compliance with ethical standards

No animals or human subjects were involved in the study.

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