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Interaction of luteolin, naringenin, and their sulfate and glucuronide conjugates with human serum albumin, cytochrome P450 (CYP2C9, CYP2C19, and CYP3A4) enzymes and organic anion transporting polypeptide (OATP1B1 and OATP2B1) transporters

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ABSTRACT

Luteolin and naringenin are flavonoids found in various foods/beverages and present in certain dietary supplements. After a high intake of these flavonoids, their sulfate and glucuronide conjugates reach micromolar concentrations in the bloodstream. Some pharmacokinetic interactions of luteolin and naringenin have been investigated in previous studies; however, only limited data are available in regard to their metabolites. In this study, we aimed to investigate the interactions of the sulfate and glucuronic acid conjugates of luteolin and naringenin with human serum albumin, cytochrome P450 (CYP2C9, 2C19, and 3A4) enzymes, and organic anion transporting polypeptide (OATP1B1 and OATP2B1) transporters. Our main findings are as follows: (1) Sulfate conjugates formed more stable complexes with albumin than the parent flavonoids. (2) Luteolin and naringenin conjugates showed no or only weak inhibitory action on the CYP enzymes examined. (3) Certain conjugates of luteolin and naringenin are potent inhibitors of OATP1B1 and/or OATP2B1 enzymes. (4) Conjugated metabolites of luteolin and naringenin may play an important role in the pharmacokinetic interactions of these flavonoids.

1. Introduction

Luteolin (LUT; Fig. 1) is a flavone aglycone. The parent compound and/or its glycosides are contained by several plants, such as carrot, pepper, onion, broccoli, cabbages, spinach, rosemary, thyme, and apple [1]. Based on previous studies, several beneficial impacts have been attributed to LUT, including anti-inflammatory [2], cardioprotective [3], neuroprotective [4], and cancer preventive effects [5]. Only a small fraction of LUT reaches the systemic circulation, typically its sulfate and glucuronide metabolites appear in the blood [1]. In a human study, luteolin-3′-*O*-sulfate has been identified as the most dominant circulating metabolite [6]. After the peroral administration of 35 mg and 50 mg LUT to healthy human volunteers, the total concentrations of LUT and its metabolites were approximately 0.5 and 1 μM, respectively [6,7]. Higher doses were also tested in animal studies, where total LUT concentrations were in the micromolar range, sometimes even exceeding the 10 μM level [1].

Naringenin (NAR; Fig. 1) is a flavanone aglycone, the parent compound and/or its glycosides typically occur in citrus fruits such as grapefruits, but they also appear in other plants, including tomatoes and figs [8]. Based on previous studies, several beneficial health effects have been attributed to NAR, including anti-inflammatory [9], cardioprotective [10], antihyperlipidemic [11], antifibrotic [12], and cancer preventive effects [13]. NAR has low oral bioavailability, its sulfate and glucuronide metabolites are the dominant forms in human blood and urine [14–16]. The consumption of 150 g orange fruit or

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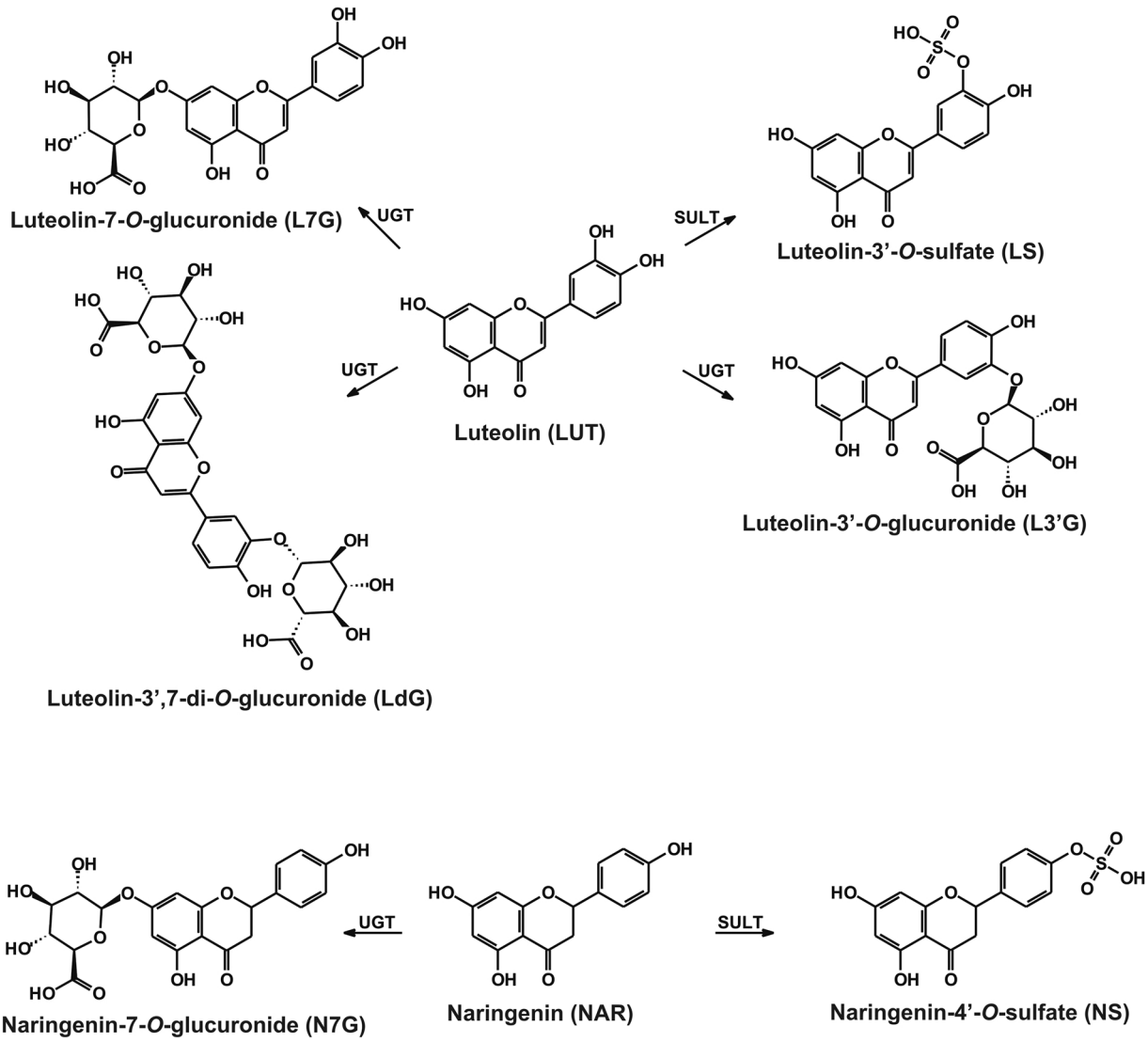

Fig. 1. Chemical structures of luteolin, luteolin-3′-*O*-sulfate, luteolin-3′-*O*-glucuronide, luteolin-7-*O*-glucuronide, luteolin-3′,7-di-*O*-glucuronide, naringenin, naringenin-4′-*O*-sulfate, and naringenin-7-*O*-glucuronide (UGT, uridine 5′-diphospho-glucuronosyltransferase; SULT, sulfotransferase).

300 g orange juice by healthy human volunteers resulted in 0.084 μM or 0.052 μM average peak plasma concentrations (C_{max}) of total NAR, respectively [16]. However, in humans, the intake of 8 mL/kg orange juice caused 0.6 μM, while the single dose of 8 mL/kg grapefruit juice led to 6 μM average C_{max} values of total NAR [17]. After the single oral administration of 150 mg and 600 mg NAR to healthy volunteers, the peak plasma concentrations of total NAR were 15.8 μM and 48.5 μM, respectively [18]. Moreover, in a rat experiment, micromolar concentrations of NAR sulfates and glucuronides were found in liver samples, where the level of glucuronides and sulfates were similar and higher than in the plasma, respectively [19].

Pharmacokinetic interactions of natural compounds with drugs are widely studied nowadays, due to the very high intake of certain nutrients through dietary supplements[20–23]. Human serum albumin (HSA) is the most abundant plasma protein in the circulation, its typical concentration is 35–50 g/L in the human blood. Sudlow's Site I (in subdomain IIA) and Sudlow's Site II (in subdomain IIIA) are the two most important drug binding sites on HSA [24,25]. Albumin transports numerous endogenous compounds, nutrients, and xenobiotics in the circulation, the formation of highly stable ligand-HSA complexes can affect the tissue distribution and/or the elimination of the bound compounds [24,25]. Furthermore, certain ligand molecules can affect the albumin binding of each other [25,26]. Cytochrome P450 (CYP) enzymes are involved in the oxidative biotransformation of most drugs [27]. CYP3A4 takes part in the biotransformation of more than 50 % of the orally administered medications, and some other CYP isoforms (e.g., CYP1A2, 2C9, 2C19, and 2D6) also have high importance in drug metabolism [28,29]. Organic anion transporting polypeptides (OATPs) are solute carrier-type membrane transporters, which are commonly involved in the tissue uptake of nutrients, drugs, and toxins [30–32]. OATP1B1 is the major hepatic drug uptake transporter, while OATP2B1 is mainly expressed by enterocytes and blood-brain barrier endothelial cells [33–35]. Previous studies demonstrated that OATPs have an important role in drug disposition, including hepatic clearance and intestinal absorption [31]. The inhibition of CYP-catalyzed elimination and/or OATP-mediated transport of drugs commonly lead to the development of pharmacokinetic interactions. For example, some coumarin and flavonoid constituents in grapefruit juice can disrupt the hepatic uptake and biotransformation of certain statins, which is one of the most widely known food-drug interactions [36,37].

Previous studies demonstrated that LUT and NAR form stable complexes with HSA [38–40]. Furthermore, both flavonoids can inhibit certain CYP enzymes [41–43] and OATP transporters [37,44,45]. On the other hand, only limited data are available on the pharmacokinetic

interactions of their sulfate and glucuronide metabolites. Our previous studies demonstrated that certain sulfate or glucuronic acid conjugates of chrysin and quercetin bound with similar or higher affinity to HSA, and produced the same or even considerably stronger inhibition of CYP enzymes and/or OATP transporters than the parent flavonoids [46–49]. Therefore, in this study, the interactions of LUT, luteolin-3′-*O*-sulfate (LS), luteolin-3′-*O*-glucuronide (L3′G), luteolin-7-*O*-glucuronide (L7G), luteolin-3′,7-di-*O*-glucuronide (LdG), NAR, naringenin-4′-*O*-sulfate (NS), and naringenin-7-*O*-glucuronide (NG) (see their chemical structures in Fig. 1) were tested with HSA, CYP (2C9, 2C19, and 3A4) enzymes, and OATP (1B1 and 2B1) transporters. Fluorescence quenching and ultrafiltration studies were employed to evaluate the stability of ligand-albumin complexes as well as to examine the displacing ability of flavonoid conjugates vs. Site I and Site II marker drugs. The inhibitory actions on CYP enzymes were tested in vitro using human recombinant CYP2C9, 2C19, and 3A4 enzymes. Finally, the effects of flavonoids on OATP1B1 and OATP2B1 transporter activity were investigated in vitro. Our results demonstrate that not only the parent flavonoids (LUT and NAR), but also their sulfate and glucuronide metabolites can interact with some of the above-listed proteins.

2. Materials and methods

2.1. Reagents

Luteolin (LUT), naringenin (NAR), human serum albumin (HSA), racemic warfarin, racemic naproxen, CypExpress™ Cytochrome P450 (CYP2C9, 2C19, and 3A4) human kits, ticlopidine, testosterone, ketoconazole, phenylbutazone, and ketoprofen were obtained from Merck (Darmstadt, Germany). Luteolin-3′-*O*-glucuronide (L3′G), luteolin-7-*O*-glucuronide (L7G), luteolin-3′,7-di-*O*-glucuronide (LdG), naringenin-7-*O*-glucuronide (N7G), diclofenac, 4′-hydroxydiclofenac, sulfaphenazole, (S)-mephenytoin, 4-hydroxymephenytoin, and 6β-hydroxytestosterone were purchased from Carbosynth (Berkshire, UK). Stock solutions (10 mM) of flavonoids were prepared in dimethyl sulfoxide (DMSO, spectroscopic grade; Fluka, Charlotte, NC, US) and stored at −20 °C. Fluorescent dyes and reagents for OATP assays, if not indicated otherwise, were purchased from Merck (Darmstadt, Germany).

Luteolin-3′-*O*-sulfate (LS) was synthesized as previously reported [50] and contained 10 % of the isomer luteolin-4′-*O*-sulfate (overall purity 99 %); the identity of the compounds was confirmed by comparison of their HPLC retention times, UV, and MS spectra with the authentic standards (Figs. S1-S3). Naringenin-4′-*O*-sulfate (NS) was prepared by an analogous procedure using the arylsulfotransferase from *Desulfitobacterium hafniense* heterologously expressed in *E. coli* [51,52]. Briefly, 205 mg of narigenin (0.75 mmol) in 5 mL of acetone was mixed with 207 mg *p*-nitrophenyl sulfate (0.8 mmol, 1.1 eq), 2 mL of the enzyme-containing cell lysate and 24 mL of 100 mM Tris-glycine buffer (pH 8.9) and incubated at 30 °C for 3 h. The reaction mixture was then partially evaporated in a rotary evaporator to remove acetone and the pH was adjusted to 7.5–7.7 with formic acid. The mixture was extracted with ethyl acetate (3 × 50 mL) to remove the reaction by-product *p*-nitrophenol and the residual starting material from the mixture (controlled by TLC; ethyl acetate/methanol/formic acid, 4:1:0.01 v/v %). The aqueous phase containing the sulfated product was completely evaporated, dissolved in 2–5 mL of 80 % aqueous methanol, and loaded onto a Sephadex LH-20 column (GE Healthcare Bio-Sciences, Uppsala, Sweden; 30 g dry weight, 3 cm i.d.) packed and equilibrated in 80 % aqueous methanol, eluting at 0.16 mL/min, 20 °C, 4 mL/fraction. Fractions were analyzed by TLC (mobile phase: ethyl acetate/methanol/formic acid, 4:1:0.01 v/v %; detection under UV light and H_2SO_4 in ethanol). The fractions containing the products were combined, evaporated, then lyophilized and characterized by HPLC, MS and NMR. The desired product, naringenin-4′-*O*-sulfate, was obtained as a greyish powder in an isolated yield of 65 mg (51 mol. %) and an HPLC purity of 99.6 %. The identity of the compound was determined by MS, ^{1}H and ^{13}C NMR analyses (see Table S1 and Figs. S4-S8).

2.2. Fluorescence spectroscopic studies

Fluorescence spectra were recorded at 25 °C employing a Hitachi F-4500 fluorimeter (Tokyo, Japan). To mimic extracellular physiological conditions, ligand-albumin interactions were tested in phosphate-buffered saline (PBS, pH 7.4). Emission spectra of HSA (2 μM) were collected in the absence and presence of flavonoids (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0, and 10 μM) using 295 nm excitation wavelength. Stern-Volmer quenching constants (K_{SV}) and binding constants (K) were determined based on the emission intensities measured at 340 nm, employing the graphical application of the Stern-Volmer equation (linear fitting) and the Hyperquad2006 program (non-linear fitting; Protonic software, Leeds, UK), respectively [53,54]. UV–vis spectra of flavonoids were also recorded in PBS (pH 7.4) using a Jasco V730 UV–vis spectrophotometer (Tokyo, Japan), then their inner-filter effects were corrected in quenching studies [54,55].

2.3. Ultrafiltration studies

The effects of flavonoids on the albumin binding of Sudlow's Site I (warfarin) and Sudlow's Site II (naproxen) markers were tested by ultrafiltration, employing the previously reported methods [56,57], without modifications. The filter units can adsorb a fraction of the site marker molecules applied. Therefore, the recovery of site markers was examined in the 0.2–1.0 μM concentration range. Since recovery values of warfarin (80.3 ± 2.1 %) and naproxen (80.4 ± 1.3 %) did not show concentration dependence, our methods are suitable for evaluate the displacement of these drugs from HSA. After ultrafiltration, the concentrations of warfarin and naproxen in the filtrate were determined by HPLC, as it has been described earlier [56,57].

2.4. CYP assays

CypExpress Cytochrome P450 (2C9, 2C19, and 3A4) human kits were applied in order to examine the in vitro inhibitory effects of LUT, NAR, and their metabolites on CYP enzymes. In each model, the FDA-recommended substrates (2C9: diclofenac; 2C19: (S)-mephenytoin; 3A4: testosterone) and inhibitors (2C9: sulfaphenazole; 2C19: ticlopidine; 3A4: ketoconazole) were used. CYP assays were performed as it has been reported [49,57], without modifications.

HPLC-UV analyses of CYP substrates and metabolites were performed applying an integrated HPLC system (Jasco, Tokyo, Japan) with an autosampler (AS-4050), a binary pump (PU-4180), and an UV detector (UV-975). Chromatograms were evaluated with the ChromNAV2 software (Jasco).

The inhibitory effects of LUT, NAR, and their metabolites on CYP2C9-catalyzed diclofenac hydroxylation were evaluated using the previously reported HPLC method [58], without modifications. Briefly, samples (20 μL) were driven through a guard column (SecurityGuard C8, 4.0 × 3.0 mm; Phenomenex, Torrance, CA, US) linked to a Teknokroma Mediterranea Sea8 (C8, 150 × 4.6 mm, 5 μm; Teknokroma, Barcelona, Spain) analytical column. The isocratic elution was performed with 1 mL/min flow rate, using phosphoric acid (6 mM) and acetonitrile (48:52 v/v %) as the mobile phase. Diclofenac and 4′-hydroxydiclofenac were detected at 275 nm.

The inhibitory effects of LUT, LS, LdG, and NG on CYP2C19-catalyzed mephenytoin hydroxylation were evaluated using the previously reported HPLC method [58], without modifications. Samples (20 μL) were driven through a guard column (SecurityGuard C8, 4.0 × 3.0 mm; Phenomenex) linked to a Luna (C8, 150 × 4.6 mm, 5 μm; Phenomenex) analytical column. The isocratic elution was performed with 1 mL/min flow rate, using sodium acetate buffer (6.9 mM, pH 4) and acetonitrile (72:28 v/v %) as the mobile phase. Diclofenac and 4′-hydroxydiclofenac were detected at 230 nm. Due to the

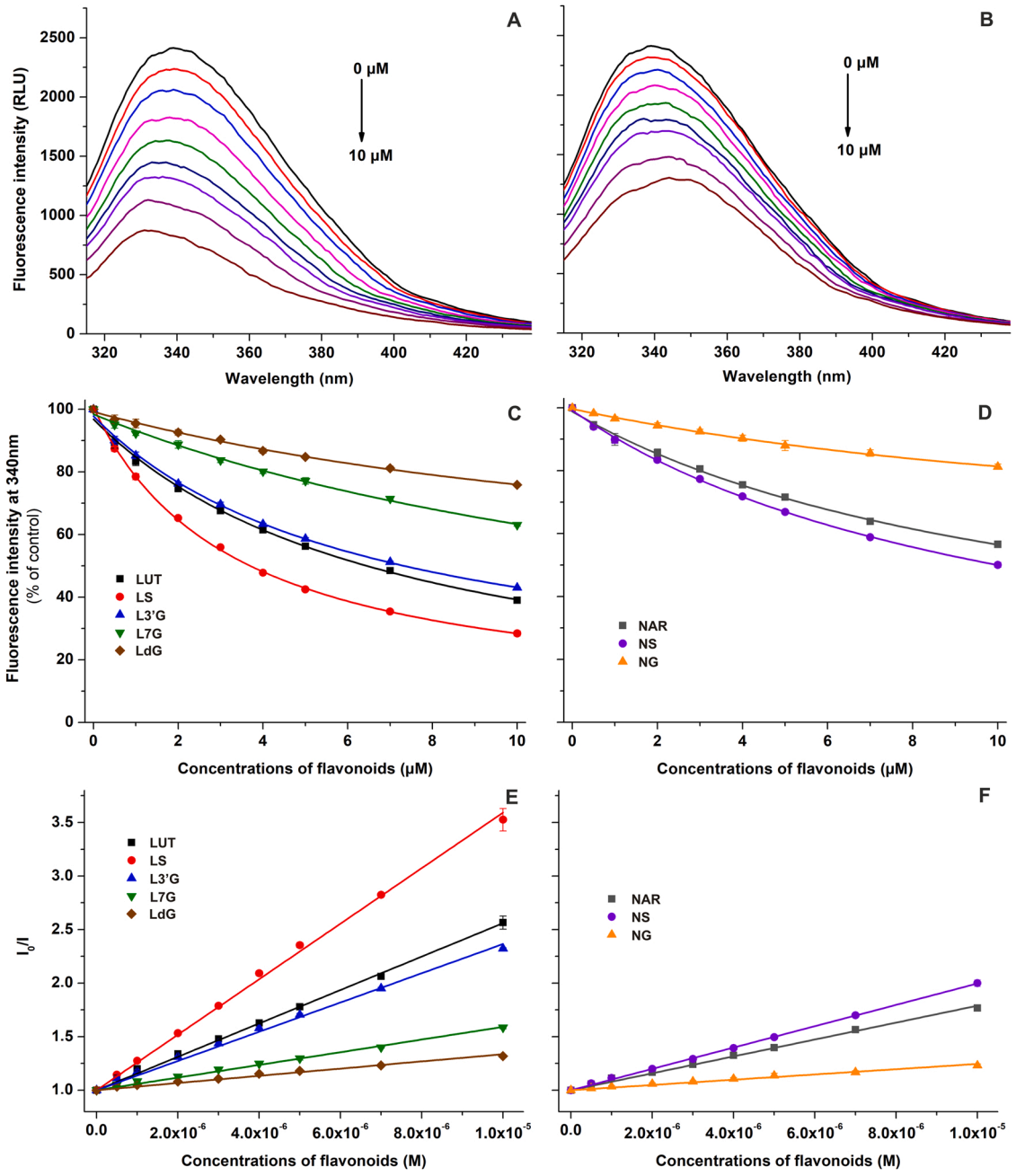

Fig. 2. Representative fluorescence emission spectra of HSA (2 μM) in the presence of increasing concentrations (0–10 μM) of luteolin (LUT; A) and naringenin (NAR; B) in PBS (pH 7.4; λ_{ex} = 295 nm). Flavonoid-induced decrease in the fluorescence emission signal of HSA at 340 nm (C and D). Stern-Volmer plots of flavonoid-HSA complexes (E and F). LS, luteolin-3′-*O*-sulfate; L3′G, luteolin-3′-*O*-glucuronide; L7G, luteolin-7-*O*-glucuronide; LdG, luteolin-3′,7-di-*O*-glucuronide; NS, naringenin-4′-*O*-sulfate; NG, naringenin-7-*O*-glucuronide.

co-elution of L3′G, L7G, NAR, and NS with the substrate or metabolite, the impacts of these compounds on CYP2C19 were tested employing the following HPLC methods.

To evaluate the inhibitory effect of NAR on CYP2C19, samples (20 μL) were driven through a guard column (SecurityGuard C18, 4.0 × 3.0 mm; Phenomenex) linked to a Kinetex EVO-C18 (C18, 150 × 4,6 mm, 5 μm; Phenomenex) analytical column. The isocratic elution was performed with 1 mL/min flow rate, using phosphoric acid solution (6 mM) and acetonitrile (70:30 v/v %) as the mobile phase. Diclofenac and 4′-hydroxydiclofenac were detected at 230 nm.

Inhibitory effects of L3′G, L7G, and NS on CYP2C19 were evaluated using the following HPLC conditions. Samples (20 μL) were driven through a guard column (SecurityGuard C8, 4.0 × 3.0 mm; Phenomenex) linked to a Teknokroma Mediterranea Sea8 (C8, 150 × 4.6 mm, 5 μm; Teknokroma) analytical column. The isocratic elution was performed with 1 mL/min flow rate, using sodium acetate buffer (10 mM, pH 4.55), acetonitrile, and methanol (63:28:14 v/v %) as the mobile phase. Diclofenac and 4′-hydroxydiclofenac were detected at 230 nm.

Inhibitory effects of LUT, NAR, and their metabolites on CYP3A4-catalyzed testosterone hydroxylation were evaluated using the previously reported HPLC method [58], without modifications. Briefly, samples (20 μL) were driven through a guard column (SecurityGuard C18, 4.0 × 3.0 mm; Phenomenex) linked to a Kinetex EVO-C18 (C18, 150 × 4,6 mm, 5 μm; Phenomenex) analytical column. The isocratic elution was performed with 1.2 mL/min flow rate, using methanol,

Table 1
Decimal logarithmic values of Stern-Volmer quenching constants (K_{SV}; unit: L/mol) and binding constants (K; unit: L/mol). LUT, luteolin; LS, luteolin-3′-*O*-sulfate; L3′G, luteolin-3′-*O*-glucuronide; L7G, luteolin-7-*O*-glucuronide; LdG, luteolin-3′,7-di-*O*-glucuronide; NAR, naringenin; NS, naringenin-4′-*O*-sulfate; NG, naringenin-7-*O*-glucuronide.

Complex	logK_{SV} (±SEM)	log$K$ (±SEM)
LUT-HSA	5.19 ± 0.01	5.29 ± 0.01
LS-HSA	5.41 ± 0.01	5.55 ± 0.01
L3′G-HSA	5.14 ± 0.01	5.23 ± 0.01
L7G-HSA	4.77 ± 0.01	4.82 ± 0.02
LdG-HSA	4.53 ± 0.01	4.56 ± 0.02
NAR-HSA	4.90 ± 0.01	4.96 ± 0.02
NS-HSA	5.00 ± 0.01	5.06 ± 0.01
NG-HSA	4.39 ± 0.04	4.41 ± 0.04

water, and acetic acid (53:46:1 v/v %) as the mobile phase. Testosterone and 6β-hydroxytestosterone were detected at 240 nm.

IC_{50} values were calculated by sigmoidal fitting (Hill1) using the Origin software (version 2018, OriginLab Corporation, Northampton, MA, US).

2.5. OATP transport inhibition assay

A431 cells overexpressing OATP1B1 or OATP2B1, or their mock transfected controls were generated previously [59], and were cultured in Dulbecco's Modified Eagle Medium (DMEM high glucose; Gibco, Thermofisher Scientific, Waltham, MA, US), supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5 % CO_2.

The interaction between flavonoids and OATP1B1 or OATP2B1 was investigated in an indirect assay using the fluorescent dye substrates, 6,8-dihydroxy-1,3-disulfopyrene (disulfopyrene) or pyranine, respectively [58,60,61]. Briefly, A431 cells (8×10^4/well) were seeded on 96-well plates in 200 μL cell culture medium one day prior to the transport measurement. Next day, the medium was removed, and the cells were washed three times with 200 μL phosphate-buffered saline (PBS, pH 7.4) and preincubated for 5 min at 37 °C with 50 μL HBSS (Hank's Balanced Salt Solution, pH 7.4; OATP1B1) or uptake buffer (pH 5.5; OATP2B1 [59]) with or without increasing concentrations of the flavonoids. The applied dye concentrations and incubation time was chosen the ensure linear transport. The transport reaction was started by the addition of 50 μL HBSS containing disulfopyrene in final concentration of 10 μM (OATP1B1) or 50 μL uptake buffer containing pyranine in final concentration of 20 μM (OATP2B1), and the cells were further incubated at 37 °C for 10 min (OATP1B1) or 15 min (OATP2B1). The reaction was stopped by removing the supernatant, after which the cells were washed three times with ice-cold PBS. Fluorescence (of 8×10^4 cells in 200 μL PBS) was determined using an Enspire plate reader (PerkinElmer, Waltham, MA, US) with excitation/emission wavelengths of 460/510 nm.

OATP-dependent transport was calculated by extracting fluorescence of corresponding controls (mock transfected cells treated the same way as OATP-expressing ones) measured in mock transfected cells. Transport activity was calculated based on the fluorescence signal in the absence of flavonoids (100 %). IC_{50} values were calculated by sigmoidal fitting (Hill1) using the Origin software (version 2018, OriginLab Corporation, Northampton, MA, US).

2.6. Statistical analyses

Data represent means and standard error of the mean (± SEM) values determined at least from three independent experiments. The statistical evaluation was established by one-way ANOVA (with Dunnett's post-hoc) test using SPSS Statistics software (IBM, Armonk, NY, US). The level of significance was set to $p < 0.05$ and $p < 0.01$.

3. Results

3.1. Interactions of LUT, NAR, and their conjugates with albumin

In fluorescence quenching studies, each flavonoid tested induced concentration-dependent decrease in the emission signal of HSA at 340 nm (Fig. 2). K_{SV} and K values showed good correlation (see logK_{SV} and log$K$ values in Table 1), both Stern-Volmer and the Hyperquad evaluations suggested the 1:1 stoichiometry of complex formation. LUT bound to the protein with higher affinity than NAR. Among the flavonoids examined, LS formed the most stable complex with HSA, while NG bound with the lowest affinity to the protein. Glucuronidation of LUT in position 7 (L7G) resulted in significant decrease in the binding affinity, then it was further reduced in the presence of a second glucuronic acid substitution in position 3′ (LdG). However, L3′G showed a binding constant similar to that of the parent flavonoid, and the presence of a sulfate substituent in the position 3′ (LS) increased the binding affinity toward HSA (Table 1). Glucuronide conjugation of NAR strongly decreased the stability of albumin complexes, while the sulfate substitution led to a slight elevation in the binding affinity.

In ultrafiltration experiments, the potential displacing effects of flavonoid metabolites were tested vs. warfarin (Site I) and naproxen (Site II). Among LUT metabolites, LS and L3′G increased the filtered fraction of warfarin (Fig. 3A), while NAR and its metabolites did not affect it (Fig. 3B). LS and L3′G also induced statistically significant elevation of naproxen concentration in the filtrate (Fig. 3C). In addition, NS increased the filtered fraction of the Site II marker, while NAR and N7G did not modified it (Fig, 3D).

3.2. Interactions of LUT, NAR, and their conjugates with CYP2C9, CYP2C19, and CYP3A4 enzymes

In the in vitro CYP assays, each positive control inhibitor induced a strong decrease in CYP-catalyzed metabolite formation (Fig. 4). First, we studied the inhibitory actions of flavonoids at four-fold (20 μM) concentrations compared to the substrates (each 5 μM). Concentration-dependent inhibitory effects were also tested when the compound produced at least 30 % inhibition in metabolite formation under the applied conditions.

CYP2C9-catalyzed diclofenac hydroxylation was not affected by the glucuronide conjugates of LUT and NAR (Fig. 4A). NS produced statistically significant but only slight (6 %) inhibition, and relatively weak (less than 20 %) inhibitory effects were demonstrated by LUT and LS. NAR was the sole compound showing strong (more than 50 %) inhibition at 20 μM concentration (Fig. 4A).

CYP2C19-catalyzed S-mephenytoin hydroxylation was inhibited by LUT and its metabolites, producing slight to moderate impacts (approximately 10–25 %) (Fig. 4B). Interestingly, the monoglucuronides (L3′G and L7G) showed weaker, while the diglucuronide (LdG) induced stronger inhibition than the parent flavonoid LUT. NAR caused more than 50 % inhibition at 20 μM concentration; nevertheless, its sulfate conjugate (NS) was only a moderate inhibitor (induced an approximately 20 % decrease in metabolite formation), and NG did not affect the enzyme (Fig. 4B).

LUT and its metabolites showed similar but only moderate (12–20 %) inhibitory actions on CYP3A4-catalyzed testosterone hydroxylation (Fig. 4C). NAR caused a major reduction in metabolite formation (60 %). However, NS and NG were only moderate inhibitors of CYP3A4, leading to approximately 25 % and 20 % decreases, respectively (Fig. 4C).

Since only NAR showed strong inhibitory effects on CYP enzymes tested, we examined the concentration-dependent impacts of this flavonoid. Even low concentrations of NAR (2.5 μM) caused statistically significant ($p < 0.01$) inhibition in each CYP assay, and the IC_{50} values were 22.3 μM, 7.4 μM, and 12.1 μM in regard to CYP2C9, CYP2C19, and CYP3A4, respectively (Fig. 5).

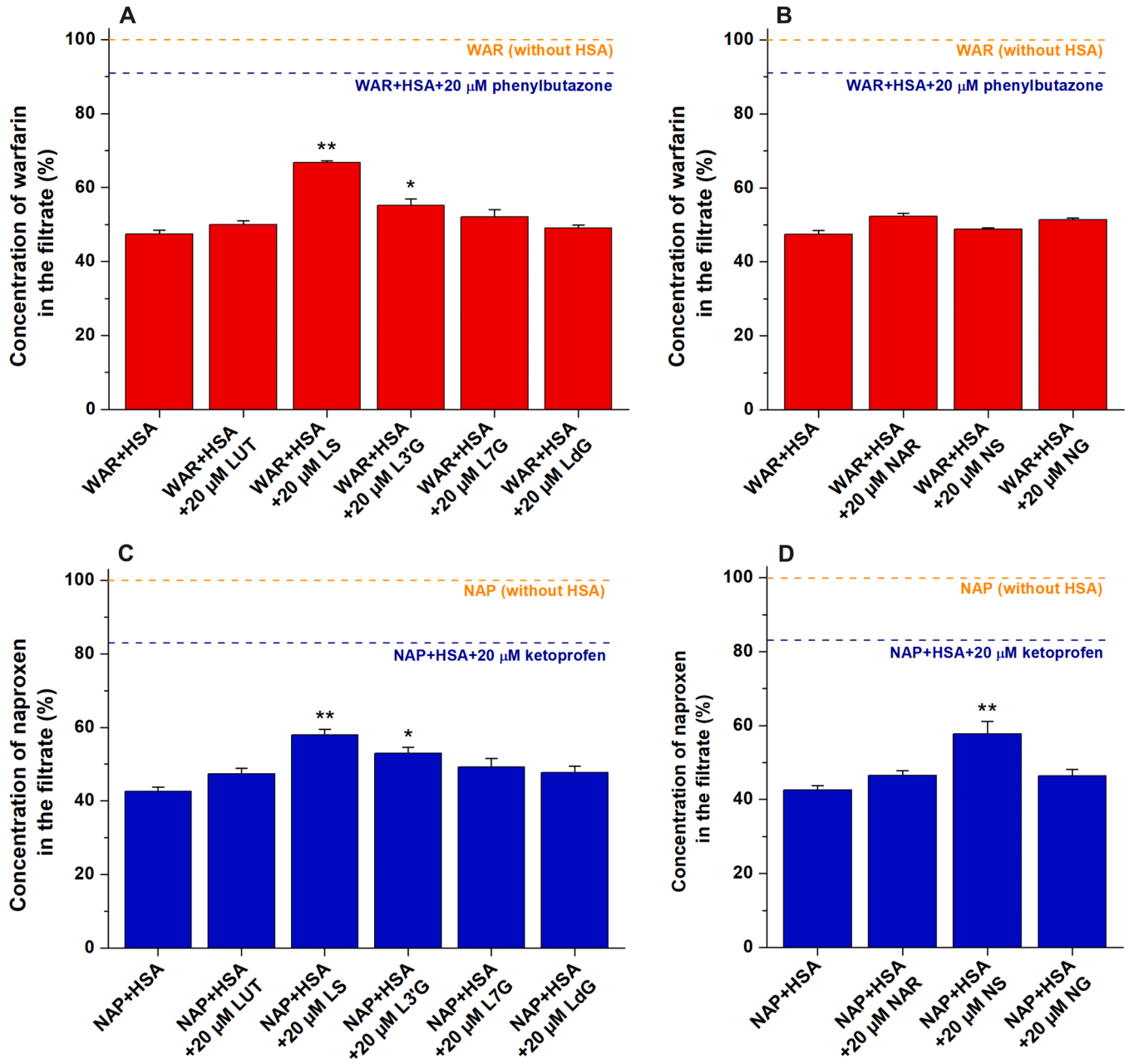

Fig. 3. Effects of flavonoids and their metabolites on the filtered fraction of Site I marker warfarin (A and B) and Site II marker naproxen (C and D). Before ultrafiltration, samples contained warfarin and HSA (1.0 and 5.0 μM, respectively) or naproxen and HSA (1.0 and 1.5 μM, respectively) without or with flavonoids (20 μM) in PBS (pH 7.4). Statistical evaluation was performed using one-way ANOVA (with Dunnett's post-hoc) test (*$p < 0.05$, **$p < 0.01$). Phenylbutazone (20 μM; Site I) and ketoprofen (20 μM; Site II) were used as positive controls (see their impacts with blue dashed lines). LUT, luteolin; LS, luteolin-3′-*O*-sulfate; L3′G, luteolin-3′-*O*-glucuronide; L7G, luteolin-7-*O*-glucuronide; LdG, luteolin-3′,7-di-*O*-glucuronide; NAR, naringenin; NS, naringenin-4′-*O*-sulfate; NG, naringenin-7-*O*-glucuronide.

3.3. Interactions of LUT, NAR, and their conjugates with OATP1B1 and OATP2B1 transporters

OATP1B1 transport activity was inhibited to a certain extent by each flavonoid tested, except NG that caused only 20 % inhibition even at 50 μM concentration (Fig. 6A and B). LUT proved to be a strong inhibitor of OATP1B1 ($IC_{50} = 0.85$ μM), while 3′-substitution of LUT with sulfate or glucuronic acid resulted in a slight increase or decrease in the inhibitory action of the metabolites, respectively. However, L7G and LdG were considerably weaker inhibitors ($IC_{50} \approx 15$ μM). NAR showed moderate inhibitory effect ($IC_{50} = 22.9$ μM) on OATP1B1 function. Its glucuronide proved to be an even weak inhibitor ($IC_{50} > 50$ μM), while NS was a three-fold stronger inhibitor ($IC_{50} = 7.66$ μM) than NAR.

In a concentration dependent fashion, OATP2B1 uptake was strongly inhibited by each flavonoid tested (Fig. 6C and D). However, NAR was approximately ten-fold weaker inhibitor of OATP2B1 than LUT. LS, L3′G, and L7G caused an inhibitory action similarly strong as LUT ($IC_{50} = 0.26$–0.42 μM), while LdG showed considerably weaker effect ($IC_{50} = 2.5$ μM). Interestingly, both sulfate and glucuronic acid conjugations strongly increased the inhibitory potency of NAR on OATP2B1, leading to three-fold (NS; $IC_{50} = 2.05$ μM) and eight-fold (NG; $IC_{50} = 0.73$ μM) stronger impacts compared to the parent flavonoid.

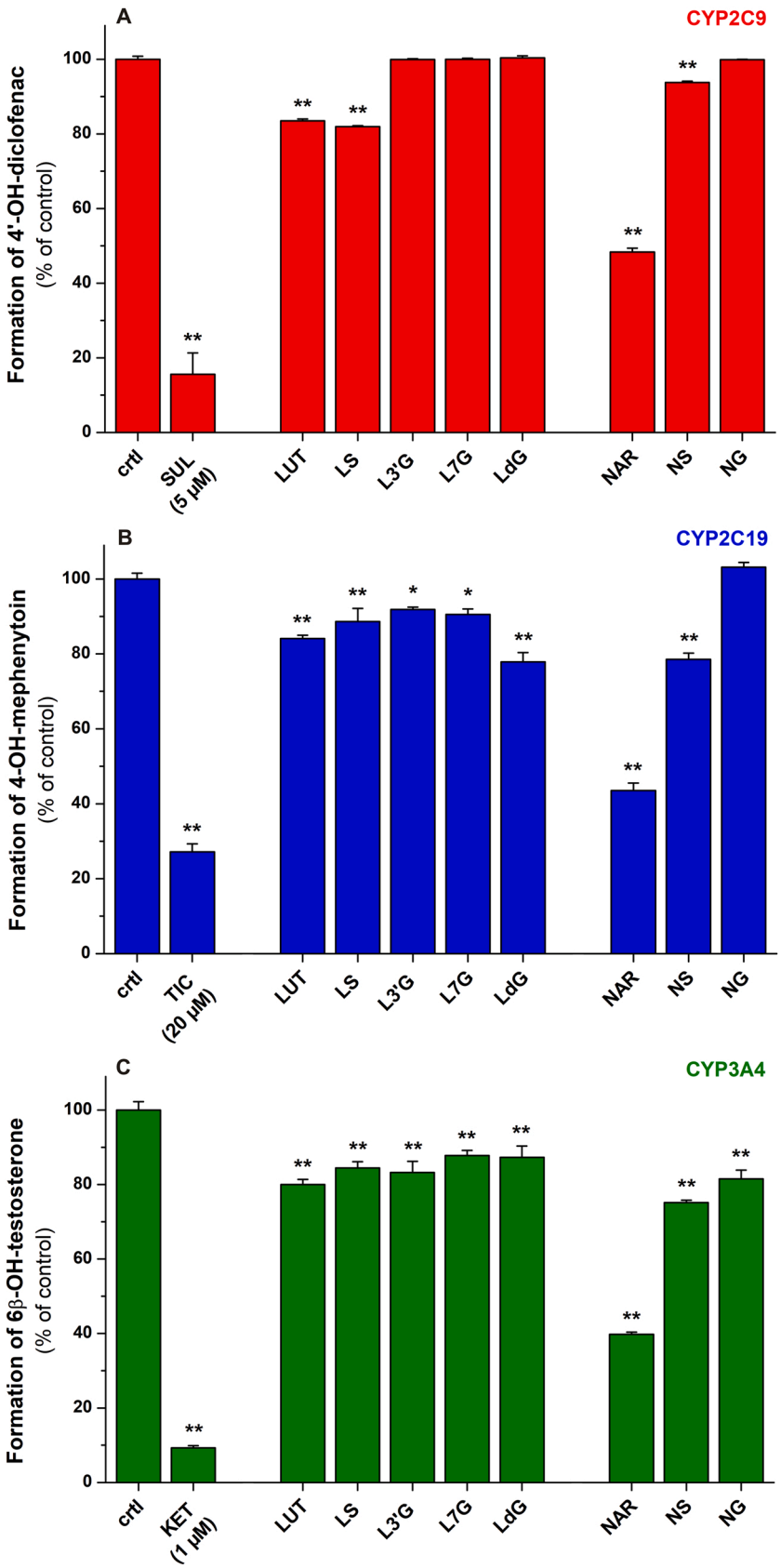

Fig. 4. Effects of luteolin (LUT), naringenin (NAR), and their sulfate/glucuronide conjugates (each 20 μM) on CYP2C9-catalyzed diclofenac hydroxylation (A), CYP2C19-catalyzed (S)-mephenytoin hydroxylation (B), and CYP3A4-catalyzed testosterone hydroxylation (C). Substrate concentrations were 5 μM in each assay. The positive controls were sulfaphenazole (SUL; CYP2C9), ticlopidine (TIC; CYP2C19), and ketoconazole (KET; CYP3A4); they caused strong decrease in metabolite formation in the corresponding assays. Statistical evaluation was performed using one-way ANOVA (with Dunnett's post-hoc) test (*$p < 0.05$, **$p < 0.01$). LS, luteolin-3′-*O*-sulfate; L3′G, luteolin-3′-*O*-glucuronide; L7G, luteolin-7-*O*-glucuronide; LdG, luteolin-3′,7-di-*O*-glucuronide; NS, naringenin-4′-*O*-sulfate; NG, naringenin-7-*O*-glucuronide.

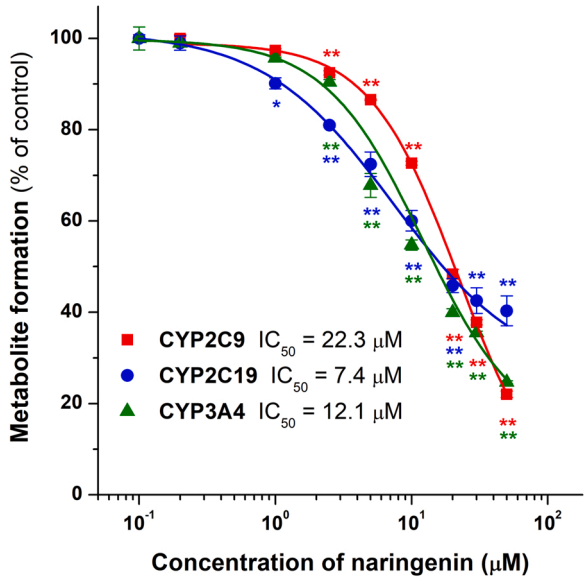

Fig. 5. Concentration-dependent inhibitory effect of naringenin on CYP2C9, CYP2C19, and CYP3A4 enzymes. Statistical evaluation was performed using one-way ANOVA (with Dunnett's post-hoc) test (*$p < 0.05$, **$p < 0.01$).

4. Discussion

There is an increasing popularity of dietary supplements, and they are widely distributed on the internet [20,21]. Besides their purported beneficial health effects, the potential pharmacokinetic and pharmacodynamic interactions of natural compounds with drugs are commonly ignored [22,23]. We did not find in vivo data in regard to the pharmacokinetic interactions of LUT. Since the number of in vitro studies is also limited, it seems to be a less studied field yet. However, pharmacokinetic interactions of NAR with CYP3A4 and OATP1B1 are well-documented and widely known [37]. The oral bioavailability of LUT or NAR is low; however, the high intake of these flavonoids results in the micromolar concentrations of their sulfate and glucuronide conjugates in the circulation [1,17]. Considering the strong interactions of sulfate and/or glucuronic acid derivatives of some flavonoids with HSA, CYP enzymes, and/or OATP transporters [46–49], it is reasonable to hypothesize that certain conjugated metabolites of LUT and/or NAR may also be involved in the potential pharmacokinetic interactions of these flavonoids.

The complex formation of a ligand molecule with HSA leads to a partial quenching effect on the fluorescence of Trp-214 (the sole tryptophane residue of the protein) [24,62]. In a concentration-dependent manner, each flavonoid decreased the emission signal of HSA at 340 nm (Fig. 2). Since the inner-filter effects were corrected, these data suggest the formation of stable flavonoid-HSA complexes [47,54]. Based on the flavonoid-induced changes in the emission signal of HSA, we determined the binding constants of the complexes formed. In the current study, the log*K* values of LUT-HSA and NAR-HSA complexes were 5.3 and 5.0, suggesting the relevant interaction of these flavonoids with the protein. The formation of highly stable complexes of LUT and NAR with HSA has also been reported in previous studies, where the binding constant of the LUT-HSA complex was 3×10^5 L/mol (log*K* ≈ 5.5) [38,39], while *K* values of NAR-HSA were in the 3×10^4 L/mol to 2×10^5 L/mol range (log*K* ≈ 4.5–5.3) [38,40]. Because of the bulky, hydrophilic structure of glucuronic acid, glucuronide conjugates of flavonoids typically form less stable complexes with HSA than the aglycones [46,47]. Therefore, the lower affinity of L7G, LdG, and NG towards the protein (compared to the parent flavonoids) was not surprising. Unexpectedly, L3′G bound to HSA with similar affinity to LUT. Furthermore, LS and NS formed more stable complexes with albumin than LUT and NAR, respectively (Table 1). This observation is in agreement with our previous results where sulfate conjugation also enhanced the interactions of quercetin and chrysin with HSA [46,47].

In ultrafiltration experiments, we tested the displacing ability of the flavonoids vs. Site I (warfarin) and Site II (naproxen) marker drugs. Since HSA (66.5 kDa) and albumin-bound molecules cannot pass through the filter unit with a 30 kDa molecular weight cut-off value, the increase in the filtered fraction of the site marker indicates its displacement from the protein [56,57]. In ultrafiltration studies, LUT, L7G, LdG, NAR, NS, and NG did not affect the free fraction of warfarin. However, L3′G and LS and (the 3′-substituted derivatives of LUT) produced slight and moderate displacement of the Site I marker, respectively (Fig. 3A). As we discussed, LS binds to HSA with higher affinity than LUT (Table 1), which is in agreement with its stronger displacing effect. However, the log*K* values of L3′G and LUT are very similar. Based on previous studies, LUT and other flavonoids occupy Site I on HSA [63–65]. However, Site I is a large cavity; therefore, the cooperative binding of warfarin with certain flavonoids is possible [66,67]. The bulkier structure of L3′G may explain its displacing effect, while LUT was not able to modify the filtered fraction of warfarin. Interestingly, LS, L3′G, and NG caused the slight to moderate displacement of the Site II marker naproxen (Fig. 3C and D). Since the direct interaction of flavonoids with Site II is not typical [63–65], it is reasonable to hypothesize the allosteric mechanism of these interactions. The above-described interactions of LUT and NAR metabolites with Site I and Site II marker drugs may have some theoretical importance; however, we noticed only their weak/moderate displacing effects vs. the ligands examined.

Only a few studies examined the interaction of LUT with CYP enzymes, where the flavonoid showed relatively strong inhibitory action on CYP2C8 and CYP1A2, while weaker inhibition was noticed on CYP3A4, 2B6, 2C9, 2C19, 2D6, and 2E1 enzymes [41,42]. In agreement with the previously reported data, our results also suggest the weak inhibition of CYP2C9, 2C19, and 3A4 enzymes by LUT (Fig. 4). Among LUT metabolites, only LS showed an inhibitory effect on CYP2C9, while each LUT conjugate was a weak or moderate inhibitor of CYP2C19 and 3A4. Considering these data, it is unlikely that LUT can strongly affect the pharmacokinetics of drugs biotransformed via these CYP enzymes. However, the combined inhibitory action of LUT and its metabolites may have some effects on the CYP-catalyzed elimination of certain drugs.

Among the flavonoids tested, NAR showed the strongest inhibitory effect on each CYP enzyme tested (Fig. 4). Few studies also reported the inhibition of CYP2C9 (diclofenac hydroxylation and 7-methoxy-4-trifluoromethylcoumarin demethylation) and CYP2C19 (3-cyano-7-ethoxycoumarin deethylation) enzymes by NAR [43,68]. Furthermore, NAR inhibited CYP3A4-catalyzed quinine 3-hydroxylation [69], testosterone hydroxylation [43], and 7-benzyloxy-4-trifluoromethylcoumarin-*O*-debenzylation [70] in human and animal liver microsomes. However, NAR conjugates produced no, weak, or moderate inhibitory effects on CYP2C9, 2C19, and/or CYP3A4 (Fig. 4). NS inhibited each enzyme tested, while NG decreased only the CYP3A4-catalyzed metabolite formation. These data suggest that sulfate and glucuronide metabolites have no relevant involvement in the NAR-induced CYP inhibition.

OATPs play a crucial role in drug disposition, hepatic clearance, and intestinal absorption. Many natural compounds can affect OATP function. Amongst these, the interactions between flavonoids, including LUT and NAR with OATPs have been extensively studied. Previous reports demonstrated that LUT can inhibit the OATP1B1-mediated uptake of dehydroepiandrosterone sulfate [71], fluvastatin [44], and 2′,7′-dichlorofluorescein [45]. More so, LUT showed inhibitory effect on 4′,5′-dibromofluorescein uptake and stimulation of 5-carboxyfluorescein transport by OATP2B1 [72]. A recent report also suggests that L3′G is a transported substrate of OATP1B1 and OATP1B3, while LUT and L7G were not transported by these carriers [73]. In our study, we confirmed the inhibitory effect of LUT on OATP1B1 and OATP2B1 activity (IC_{50} values were 0.85 μM and 0.42 μM, respectively). Previous studies

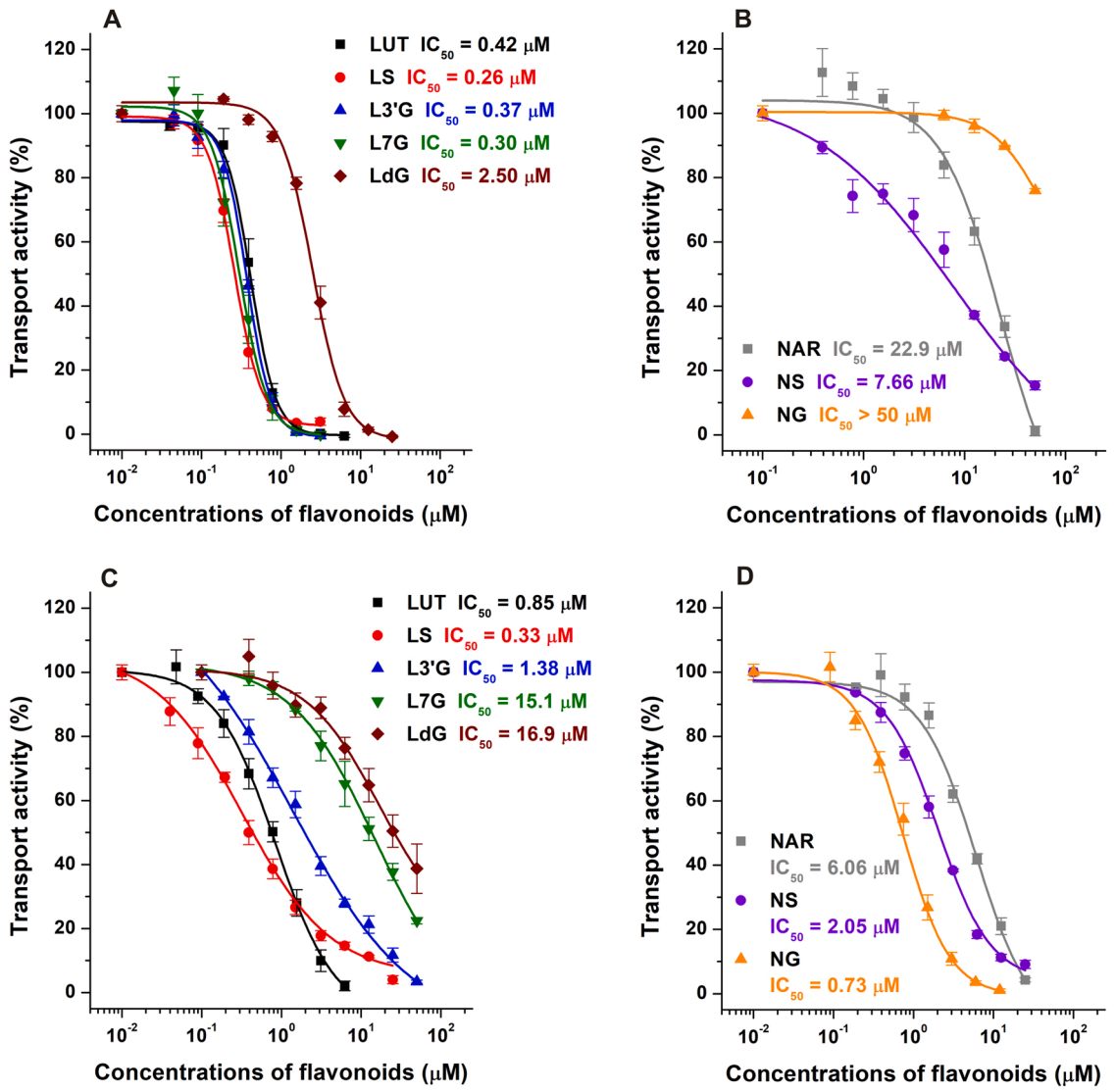

Fig. 6. Concentration-dependent inhibitory effects of luteolin (LUT), naringenin (NAR), and their conjugates on OATP1B1 and OATP2B1 functions. Disulfopyranine (OATP1B1) and pyranine (OATP2B1) uptake was determined in A431 cells in the absence or presence of increasing concentrations of the flavonoids. Statistical evaluation was performed using one-way ANOVA (with Dunnett's post-hoc) test. OATP1B1: statistically significant ($p < 0.01$) inhibition was induced by 0.4 μM LUT, 0.1 μM LS, 0.4 μM L3′G, 3.1 μM L7G, 6.3 μM LdG, 12.5 μM NAR, 0.8 μM NS, and 50 μM NG. OATP2B1: statistically significant ($p < 0.01$) inhibition was induced by 0.4 μM LUT, 0.2 μM LS, 0.2 μM L3′G, 0.4 μM L7G, 1.6 μM LdG, 3.1 μM NAR, 0.8 μM NS, and 0.4 μM NG. LS, luteolin-3′-*O*-sulfate; L3′G, luteolin-3′-*O*-glucuronide; L7G, luteolin-7-*O*-glucuronide; LdG, luteolin-3′,7-di-*O*-glucuronide; NS, naringenin-4′-*O*-sulfate; NG, naringenin-7-*O*-glucuronide.

demonstrated that certain LUT conjugates are excreted into the bile and take part in enterohepatic circulation [6,74,75], suggesting that not only the aglycone but LUT metabolites may also affect the OATP2B1-mediated absorption of drugs. In addition, we demonstrated here our novel observation that sulfate and glucuronide conjugates of LUT can inhibit hepatic and intestinal OATPs, 1B1 and 2B1. Considering the possible plasma concentrations and the strong inhibitory potency of certain LUT metabolites, it is reasonable to hypothesize that the high intake of LUT can lead to the in vivo inhibition of OATP1B1 and OATP2B1 transporters.

Similar to LUT, the interaction of NAR with OATPs has also been investigated before. NAR showed inhibitory effects on the OATP1B1-mediated uptake of dehydroepiandrosterone sulfate [71], fluvastatin [44], and 2′,7′-dichlorofluorescein [45]. Furthermore, NAR also inhibited the OATP1B1- and OATP1B3-mediated sulfobromophthalein [30] as well as the OATP1A2- and OATP2B1-mediated estrone-3-sulfate transport [36,76,77]. A recent report suggests that naringenin acts as an inhibitor of 4′,5′-dibromofluorescein uptake mediated by OATP2B1 [72], while another study described that NAR is not taken up by this transporter [78]. Herein, we demonstrated that not only NAR, but also its sulfate and glucuronide conjugates can inhibit OATP1B1 and OATP2B1 function. Moreover, we found that NS is a stronger inhibitor of these carriers than the parent flavonoid. In addition, NG is a highly potent inhibitor of OATP2B1 (IC_{50} = 0.73 μM). Considering the high concentrations of NAR conjugates in the circulation and the above-listed novel findings, it is reasonable to hypothesize that the metabolites play a major role in the in vivo inhibition of these OATPs after the high intake of NAR. As it has been reported, both NAR sulfate and glucuronide are excreted into the intestines and take part in enterohepatic circulation [79–81]; therefore, it is reasonable to hypothesize that NAR metabolites can also inhibit the OATP2B1-mediated absorption of certain drugs.

This study further supports our previous findings [46–49] that pharmacokinetic interactions of conjugated flavonoid metabolites should be seriously considered: (1) Sulfate metabolites of chrysin, quercetin, luteolin, and naringenin formed more stable complexes with HSA than the parent flavonoids. Furthermore, in this study, L3′G also

showed strong interaction with albumin. (2) Sulfate metabolites of flavonoids commonly show similar or even stronger inhibitory actions on CYP enzymes than the aglycones. (3) Typically sulfate conjugates (and sometimes glucuronides) are similar or even considerably stronger inhibitors of certain OATP transporters than the parent flavonoids.

5. Conclusions

In summary, the interactions of LUT, NAR, and their sulfate/glucuronide conjugates were examined with HSA, CYP (2C9, 2C19, and 3A4) enzymes, and OATP (1B1 and 2B1) transporters employing in vitro models. LS and NS formed more stable complexes with HSA than LUT and NAR, respectively. NAR exerted strong inhibition on the CYP enzymes tested, conjugates of LUT and NAR showed no or only weak inhibitory actions. LUT, LS, and L3′G were potent inhibitors of OATP1B1, then NS, L7G, LdG, and NAR caused moderately strong inhibition, while NG showed only weak effect. LUT, LS, L3′G, L7G, and NG proved to be potent inhibitors of OATP2B1, while NS, LdG, and NAR had moderately strong inhibitory effects. These observations suggest that conjugated metabolites of LUT and NAR may be involved in the pharmacokinetic interactions of these flavonoids. Our study also underlines that the high intake of LUT and/or NAR together with certain medications may affect pharmacotherapy.

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CRediT authorship contribution statement

Hana Kaci: Formal analysis, Investigation; Slávka Bodnárová: Formal analysis, Investigation; **Eszter Fliszár-Nyúl:** Formal analysis, Investigation; **Beáta Lemli:** Formal analysis, Investigation; **Éva Bakos:** Formal analysis, Investigation; **Helena Pelantová:** Formal analysis, Investigation; **Kateřina Valentová:** Funding acquisition, Methodology, Validation, Writing - original draft; **Csilla Özvegy-Laczka:** Conceptualization, Funding acquisition, Methodology, Validation, Writing - original draft; **Miklós Poór:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing - original draft.

Conflict of Interest statement

The authors declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.114078.

References

[1] Y. Taheri, J. Sharifi-Rad, G. Antika, Y.B. Yılmaz, T.B. Tumer, S. Abuhamdah, S. Chandra, S. Saklani, C.S. Kılıç, S. Sestito, S.D. Daştan, M. Kumar, M.M. Alshehri, S. Rapposelli, N. Cruz-Martins, W.C. Cho, Paving luteolin therapeutic potentialities and agro-food-pharma applications: emphasis on in vivo pharmacological effects and bioavailability traits, Oxid. Med. Cell. Longev. 2021 (2021) 1–20, https://doi.org/10.1155/2021/1987588.

[2] S. Mahdiani, N. Omidkhoda, S. Heidari, A.W. Hayes, G. Karimi, Protective effect of luteolin against chemical and natural toxicants by targeting NF-κB pathway, BioFactors 48 (2022) 744–762, https://doi.org/10.1002/biof.1876.

[3] Q. Pan, Y. Liu, W. Ma, R. Kan, H. Zhu, D. Li, Cardioprotective effects and possible mechanisms of luteolin for myocardial ischemia-reperfusion injury: a systematic review and meta-analysis of preclinical evidence, Front. Cardiovasc. Med. 9 (2022), 685998, https://doi.org/10.3389/fcvm.2022.685998.

[4] D. Kempuraj, R. Thangavel, D.D. Kempuraj, M.E. Ahmed, G.P. Selvakumar, S. P. Raikwar, S.A. Zaheer, S.S. Iyer, R. Govindarajan, P.N. Chandrasekaran, A. Zaheer, Neuroprotective effects of flavone luteolin in neuroinflammation and neurotrauma, BioFactors 47 (2021) 190–197, https://doi.org/10.1002/biof.1687.

[5] S.A. Ganai, F.A. Sheikh, Z.A. Baba, M.A. Mir, M.A. Mantoo, M.A. Yatoo, Anticancer activity of the plant flavonoid luteolin against preclinical models of various cancers and insights on different signalling mechanisms modulated, Phytother. Res. 35 (2021) 3509–3532, https://doi.org/10.1002/ptr.7044.

[6] N. Hayasaka, N. Shimizu, T. Komoda, S. Mohri, T. Tsushida, T. Eitsuka, T. Miyazawa, K. Nakagawa, Absorption and metabolism of luteolin in rats and humans in relation to in vitro anti-inflammatory effects, J. Agric. Food Chem. 66 (2018) 11320–11329, https://doi.org/10.1021/acs.jafc.8b03273.

[7] S.M. Wittemer, M. Ploch, T. Windeck, S.C. Müller, B. Drewelow, H. Derendorf, M. Veit, Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of Artichoke leaf extracts in humans, Phytomedicine 12 (2005) 28–38, https://doi.org/10.1016/j.phymed.2003.11.002.

[8] B. Salehi, P. Fokou, M. Sharifi-Rad, P. Zucca, R. Pezzani, N. Martins, J. Sharifi-Rad, The therapeutic potential of naringenin: a review of clinical trials, Pharmaceuticals 12 (2019) 11, https://doi.org/10.3390/ph12010011.

[9] M. Alimohammadi, R.N. Mohammad, A. Rahimi, F. Faramarzi, R. Alizadeh-Navaei, A. Rafiei, The effect of immunomodulatory properties of naringenin on the inhibition of inflammation and oxidative stress in autoimmune disease models: a systematic review and meta-analysis of preclinical evidence, Inflamm. Res. (2022), https://doi.org/10.1007/s00011-022-01599-7.

[10] R. Heidary Moghaddam, Z. Samimi, S.Z. Moradi, P.J. Little, S. Xu, M.H. Farzaei, Naringenin and naringin in cardiovascular disease prevention: A preclinical review, Eur. J. Pharmacol. 887 (2020), 173535, https://doi.org/10.1016/j.ejphar.2020.173535.

[11] Y. Yang, M. Trevethan, S. Wang, L. Zhao, Beneficial effects of citrus flavanones naringin and naringenin and their food sources on lipid metabolism: An update on bioavailability, pharmacokinetics, and mechanisms, J. Nutr. Biochem. 104 (2022), 108967, https://doi.org/10.1016/j.jnutbio.2022.108967.

[12] Y. Du, J. Ma, Y. Fan, X. Wang, S. Zheng, J. Feng, J. Li, Z. Fan, G. Li, Q. Ye, Naringenin: a promising therapeutic agent against organ fibrosis, Oxid. Med. Cell. Longev. 2021 (2021) 1–13, https://doi.org/10.1155/2021/1210675.

[13] F. Faramarzi, M. Alimohammadi, A. Rahimi, R. Alizadeh-Navaei, R.J. Shakib, A. Rafiei, Naringenin induces intrinsic and extrinsic apoptotic signaling pathways in cancer cells: a systematic review and meta-analysis of in vitro and in vivo data, Nutr. Res. 105 (2022) 33–52, https://doi.org/10.1016/j.nutres.2022.05.003.

[14] R. Joshi, Y.A. Kulkarni, S. Wairkar, Pharmacokinetic, pharmacodynamic and formulations aspects of Naringenin: an update, Life Sci. 215 (2018) 43–56, https://doi.org/10.1016/j.lfs.2018.10.066.

[15] J. Zhang, J.S. Brodbelt, Screening flavonoid metabolites of naringin and narirutin in urine after human consumption of grapefruit juice by LC-MS and LC-MS/MS, Analyst 129 (2004) 1227, https://doi.org/10.1039/b412577k.

[16] G.M. Brett, W. Hollands, P.W. Needs, B. Teucher, J.R. Dainty, B.D. Davis, J. S. Brodbelt, P.A. Kroon, Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion, Br. J. Nutr. 101 (2009) 664–675, https://doi.org/10.1017/S000711450803081X.

[17] I. Erlund, E. Meririnne, G. Alfthan, A. Aro, Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice, J. Nutr. 131 (2001) 235–241, https://doi.org/10.1093/jn/131.2.235.

[18] C.J. Rebello, R.A. Beyl, J.J.L. Lertora, F.L. Greenway, E. Ravussin, D.M. Ribnicky, A. Poulev, B.J. Kennedy, H.F. Castro, S.R. Campagna, A.A. Coulter, L.M. Redman, Safety and pharmacokinetics of naringenin: a randomized, controlled, single-ascending-dose clinical trial, Diabetes Obes. Metab. 22 (2020) 91–98, https://doi.org/10.1111/dom.13868.

[19] S.-P. Lin, Y.-C. Hou, S.-Y. Tsai, M.-J. Wang, P.-D.L. Chao, Tissue distribution of naringenin conjugated metabolites following repeated dosing of naringin to rats, BioMedicine 4 (2014) 16, https://doi.org/10.7603/s40681-014-0016-z.

[20] R.G. Vida, A. Fittler, A. Somogyi-Végh, M. Poór, Dietary quercetin supplements: assessment of online product informations and quantitation of quercetin in the products by high-performance liquid chromatography, Phytother. Res. 33 (2019) 1912–1920, https://doi.org/10.1002/ptr.6382.

[21] L. Baudischova, J. Straznicka, J. Pokladnikova, L. Jahodar, The quality of information on the internet relating to top-selling dietary supplements in the Czech Republic, Int. J. Clin. Pharm. 40 (2018) 183–189, https://doi.org/10.1007/s11096-017-0564-x.

[22] A.I. Geller, N. Shehab, N.J. Weidle, M.C. Lovegrove, B.J. Wolpert, B.B. Timbo, R. P. Mozersky, D.S. Budnitz, Emergency department visits for adverse events related to dietary supplements, N. Engl. J. Med. 373 (2015) 1531–1540, https://doi.org/10.1056/NEJMsa1504267.

[23] V.M. Wheatley, J. Spink, Defining the public health threat of dietary supplement fraud, Compr. Rev. Food Sci. Food Saf. 12 (2013) 599–613, https://doi.org/10.1111/1541-4337.12033.

[24] G. Fanali, A. di Masi, V. Trezza, M. Marino, M. Fasano, P. Ascenzi, Human serum albumin: from bench to bedside, Mol. Asp. Med. 33 (2012) 209–290, https://doi.org/10.1016/j.mam.2011.12.002.
[25] S. Schmidt, D. Gonzalez, H. Derendorf, Significance of protein binding in pharmacokinetics and pharmacodynamics, J. Pharm. Sci. 99 (2010) 1107–1122, https://doi.org/10.1002/jps.21916.
[26] K. Yamasaki, V.T.G. Chuang, T. Maruyama, M. Otagiri, Albumin–drug interaction and its clinical implication, Biochim. Biophys. Acta BBA - Gen. Subj. 1830 (2013) 5435–5443, https://doi.org/10.1016/j.bbagen.2013.05.005.
[27] B. Meunier, S.P. de Visser, S. Shaik, Mechanism of oxidation reactions catalyzed by cytochrome P450 enzymes, Chem. Rev. 104 (2004) 3947–3980, https://doi.org/10.1021/cr020443g.
[28] G.R. Wilkinson, Drug metabolism and variability among patients in drug response, N. Engl. J. Med. 352 (2005) 2211–2221, https://doi.org/10.1056/NEJMra032424.
[29] A.M. McDonnell, C.H. Dang, Basic review of the cytochrome P450 system, J. Adv. Pract. Oncol. 4 (2013), https://doi.org/10.6004/jadpro.2013.4.4.7.
[30] K. Mandery, B. Balk, K. Bujok, I. Schmidt, M.F. Fromm, H. Glaeser, Inhibition of hepatic uptake transporters by flavonoids, Eur. J. Pharm. Sci. 46 (2012) 79–85, https://doi.org/10.1016/j.ejps.2012.02.014.
[31] Y. Shitara, K. Maeda, K. Ikejiri, K. Yoshida, T. Horie, Y. Sugiyama, Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption: clinical significance of OATPs in drug disposition, Biopharm. Drug Dispos. 34 (2013) 45–78, https://doi.org/10.1002/bdd.1823.
[32] K. Letschert, H. Faulstich, D. Keller, D. Keppler, Molecular characterization and inhibition of amanitin uptake into human hepatocytes, Toxicol. Sci. 91 (2006) 140–149, https://doi.org/10.1093/toxsci/kfj141.
[33] J. König, Y. Cui, A.T. Nies, D. Keppler, A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane, Am. J. Physiol. -Gastrointest. Liver Physiol. 278 (2000) G156–G164, https://doi.org/10.1152/ajpgi.2000.278.1.G156.
[34] B.L. Urquhart, R.B. Kim, Blood−brain barrier transporters and response to CNS-active drugs, Eur. J. Clin. Pharmacol. 65 (2009) 1063–1070, https://doi.org/10.1007/s00228-009-0714-8.
[35] T. Nakanishi, I. Tamai, Genetic polymorphisms of OATP transporters and their impact on intestinal absorption and hepatic disposition of drugs, Drug Metab. Pharmacokinet. 27 (2012) 106–121, https://doi.org/10.2133/dmpk.DMPK-11-RV-099.
[36] E.J. Johnson, C.S. Won, K. Köck, M.F. Paine, Prioritizing pharmacokinetic drug interaction precipitants in natural products: application to OATP inhibitors in grapefruit juice: prioritizing OATP2B1 inhibitors in GFJ, Biopharm. Drug Dispos. 38 (2017) 251–259, https://doi.org/10.1002/bdd.2061.
[37] J. Zechner, S.M. Britza, R. Farrington, R.W. Byard, I.F. Musgrave, Flavonoid-statin interactions causing myopathy and the possible significance of OATP transport, CYP450 metabolism and mevalonate synthesis, Life Sci. 291 (2022), 119975, https://doi.org/10.1016/j.lfs.2021.119975.
[38] J. Xiao, Y. Zhao, H. Wang, Y. Yuan, F. Yang, C. Zhang, K. Yamamoto, Noncovalent interaction of dietary polyphenols with common human plasma proteins, J. Agric. Food Chem. 59 (2011) 10747–10754, https://doi.org/10.1021/jf2029829.
[39] S. Sarmah, S. Pahari, V.K. Belwal, M. Jana, A. Singha Roy, Elucidation of molecular interaction of bioactive flavonoid luteolin with human serum albumin and its glycated analogue using multi-spectroscopic and computational studies, J. Mol. Liq. 318 (2020), 114147, https://doi.org/10.1016/j.molliq.2020.114147.
[40] F. Yazdani, B. Shareghi, S. Farhadian, L. Momeni, Structural insights into the binding behavior of flavonoids naringenin with Human Serum Albumin, J. Mol. Liq. 349 (2022), 118431, https://doi.org/10.1016/j.molliq.2021.118431.
[41] L. Cao, A. Kwara, D.J. Greenblatt, Metabolic interactions between acetaminophen (paracetamol) and two flavonoids, luteolin and quercetin, through in-vitro inhibition studies, J. Pharm. Pharm. 69 (2017) 1762–1772, https://doi.org/10.1111/jphp.12812.
[42] L. Quintieri, P. Palatini, A. Nassi, P. Ruzza, M. Floreani, Flavonoids diosmetin and luteolin inhibit midazolam metabolism by human liver microsomes and recombinant CYP 3A4 and CYP3A5 enzymes, Biochem. Pharmacol. 75 (2008) 1426–1437, https://doi.org/10.1016/j.bcp.2007.11.012.
[43] Y. Kimura, H. Ito, R. Ohnishi, T. Hatano, Inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity, Food Chem. Toxicol. 48 (2010) 429–435, https://doi.org/10.1016/j.fct.2009.10.041.
[44] X. Fan, J. Bai, M. Hu, Y. Xu, S. Zhao, Y. Sun, B. Wang, J. Hu, Y. Li, Drug interaction study of flavonoids toward OATP1B1 and their 3D structure activity relationship analysis for predicting hepatoprotective effects, Toxicology 437 (2020), 152445, https://doi.org/10.1016/j.tox.2020.152445.
[45] Y. Xiang, S. Liu, J. Yang, Z. Wang, H. Zhang, C. Gui, Investigation of the interactions between flavonoids and human organic anion transporting polypeptide 1B1 using fluorescent substrate and 3D-QSAR analysis, Biochim. Biophys. Acta BBA - Biomembr. 1862 (2020), 183210, https://doi.org/10.1016/j.bbamem.2020.183210.
[46] M. Poór, G. Boda, P.W. Needs, P.A. Kroon, B. Lemli, T. Bencsik, Interaction of quercetin and its metabolites with warfarin: displacement of warfarin from serum albumin and inhibition of CYP2C9 enzyme, Biomed. Pharmacother. 88 (2017) 574–581, https://doi.org/10.1016/j.biopha.2017.01.092.
[47] V. Mohos, E. Fliszár-Nyúl, G. Schilli, C. Hetényi, B. Lemli, S. Kunsági-Máté, B. Bognár, M. Poór, Interaction of chrysin and its main conjugated metabolites chrysin-7-sulfate and chrysin-7-glucuronide with serum albumin, Int. J. Mol. Sci. 19 (2018) 4073, https://doi.org/10.3390/ijms19124073.
[48] V. Mohos, E. Fliszár-Nyúl, O. Ungvári, K. Kuffa, P.W. Needs, P.A. Kroon, Á. Telbisz, C. Özvegy-Laczka, M. Poór, Inhibitory effects of quercetin and its main methyl, sulfate, and glucuronic acid conjugates on cytochrome P450 enzymes, and on OATP, BCRP and MRP2 transporters, Nutrients 12 (2020) 2306, https://doi.org/10.3390/nu12082306.
[49] V. Mohos, E. Fliszár-Nyúl, O. Ungvári, É. Bakos, K. Kuffa, T. Bencsik, B.Z. Zsidó, C. Hetényi, Á. Telbisz, C. Özvegy-Laczka, M. Poór, Effects of chrysin and its major conjugated metabolites chrysin-7-sulfate and chrysin-7-glucuronide on cytochrome P450 enzymes and on OATP, P-gp, BCRP, and MRP2 transporters, Drug Metab. Dispos. 48 (2020) 1064–1073, https://doi.org/10.1124/dmd.120.000085.
[50] K. Káňová, L. Petrásková, H. Pelantová, Z. Rybková, K. Malachová, J. Cvačka, V. Křen, K. Valentová, Sulfated metabolites of luteolin, myricetin, and ampelopsin: chemoenzymatic preparation and biophysical properties, J. Agric. Food Chem. 68 (2020) 11197–11206, https://doi.org/10.1021/acs.jafc.0c03997.
[51] K. Brodsky, K. Káňová, D. Konvalinková, K. Slámová, H. Pelantová, K. Valentová, P. Bojarová, V. Křen, L. Petrásková, Bacterial aryl sulfotransferases in selective and sustainable sulfation of biologically active compounds using novel sulfate donors, ChemSusChem 15 (2022), https://doi.org/10.1002/cssc.202201253.
[52] L. Petrásková, K. Káňová, K. Brodsky, A. Hetman, B. Petránková, H. Pelantová, V. Křen, K. Valentová, Sulfated phenolic substances: preparation and optimized HPLC analysis, Int. J. Mol. Sci. 23 (2022) 5743, https://doi.org/10.3390/ijms23105743.
[53] M. Poór, G. Boda, V. Mohos, M. Kuzma, M. Bálint, C. Hetényi, T. Bencsik, Pharmacokinetic interaction of diosmetin and silibinin with other drugs: Inhibition of CYP2C9-mediated biotransformation and displacement from serum albumin, Biomed. Pharmacother. 102 (2018) 912–921, https://doi.org/10.1016/j.biopha.2018.03.146.
[54] Z. Faisal, B. Lemli, D. Szerencsés, S. Kunsági-Máté, M. Bálint, C. Hetényi, M. Kuzma, M. Mayer, M. Poór, Interactions of zearalenone and its reduced metabolites α-zearalenol and β-zearalenol with serum albumins: species differences, binding sites, and thermodynamics, Mycotoxin Res. 34 (2018) 269–278, https://doi.org/10.1007/s12550-018-0321-6.
[55] T. Hu, Y. Liu, Probing the interaction of cefodizime with human serum albumin using multi-spectroscopic and molecular docking techniques, J. Pharm. Biomed. Anal. 107 (2015) 325–332, https://doi.org/10.1016/j.jpba.2015.01.010.
[56] E. Fliszár-Nyúl, B. Lemli, S. Kunsági-Máté, L. Dellafiora, C. Dall'Asta, G. Cruciani, G. Pethő, M. Poór, Interaction of mycotoxin alternariol with serum albumin, Int. J. Mol. Sci. 20 (2019) 2352, https://doi.org/10.3390/ijms20092352.
[57] V. Mohos, E. Fliszár-Nyúl, B. Lemli, B.Z. Zsidó, C. Hetényi, P. Mladěnka, P. Horký, M. Pour, M. Poór, Testing the pharmacokinetic interactions of 24 colonic flavonoid metabolites with human serum albumin and cytochrome P450 enzymes, Biomolecules 10 (2020) 409, https://doi.org/10.3390/biom10030409.
[58] M. Poór, H. Kaci, S. Bodnárová, V. Mohos, E. Fliszár-Nyúl, S. Kunsági-Máté, C. Özvegy-Laczka, B. Lemli, Interactions of resveratrol and its metabolites (resveratrol-3-sulfate, resveratrol-3-glucuronide, and dihydroresveratrol) with serum albumin, cytochrome P450 enzymes, and OATP transporters, Biomed. Pharmacother. 151 (2022), 113136, https://doi.org/10.1016/j.biopha.2022.113136.
[59] I. Patik, V. Székely, O. Német, Á. Szepesi, N. Kucsma, G. Várady, G. Szakács, É. Bakos, C. Özvegy-Laczka, Identification of novel cell-impermeant fluorescent substrates for testing the function and drug interaction of organic anion-transporting polypeptides, OATP1B1/1B3 and 2B1, Sci. Rep. 8 (2018) 2630, https://doi.org/10.1038/s41598-018-20815-1.
[60] V. Székely, I. Patik, O. Ungvári, Á. Telbisz, G. Szakács, É. Bakos, C. Özvegy-Laczka, Fluorescent probes for the dual investigation of MRP2 and OATP1B1 function and drug interactions, Eur. J. Pharm. Sci. 151 (2020), 105395, https://doi.org/10.1016/j.ejps.2020.105395.
[61] O. Ungvári, L. Király, É. Bakos, C. Özvegy-Laczka, 8-acetoxy-trisulfopyrene as the first activatable fluorogenic probe for add-and-read assessment of Organic anion-transporting polypeptides, OATP1B1, OATP1B3, and OATP2B1, FASEB J. 35 (2021), e21863, https://doi.org/10.1096/fj.202100648R.
[62] M. van de Weert, L. Stella, Fluorescence quenching and ligand binding: a critical discussion of a popular methodology, J. Mol. Struct. 998 (2011) 144–150, https://doi.org/10.1016/j.molstruc.2011.05.023.
[63] C. Dufour, O. Dangles, Flavonoid–serum albumin complexation: determination of binding constants and binding sites by fluorescence spectroscopy, Biochim. Biophys. Acta BBA - Gen. Subj. 1721 (2005) 164–173, https://doi.org/10.1016/j.bbagen.2004.10.013.
[64] S. Pal, C. Saha, A review on structure–affinity relationship of dietary flavonoids with serum albumins, J. Biomol. Struct. Dyn. 32 (2014) 1132–1147, https://doi.org/10.1080/07391102.2013.811700.
[65] H. Liu, W. Bao, H. Ding, J. Jang, G. Zou, Binding modes of flavones to human serum albumin: insights from experimental and computational studies, J. Phys. Chem. B. 114 (2010) 12938–12947, https://doi.org/10.1021/jp102053x.
[66] F. Zsila, Z. Bikádi, M. Simonyi, Probing the binding of the flavonoid, quercetin to human serum albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods, Biochem. Pharmacol. 65 (2003) 447–456, https://doi.org/10.1016/S0006-2952(02)01521-6.
[67] H. Rimac, C. Dufour, Ž. Debeljak, B. Zorc, M. Bojić, Warfarin and flavonoids do not share the same binding region in binding to the IIA subdomain of human serum albumin, Molecules 22 (2017) 1153, https://doi.org/10.3390/molecules22071153.
[68] W.J. Lu, V. Ferlito, C. Xu, D.A. Flockhart, S. Caccamese, Enantiomers of naringenin as pleiotropic, stereoselective inhibitors of cytochrome P450 isoforms, Chirality 23 (2011) 891–896, https://doi.org/10.1002/chir.21005.
[69] P.-C. Ho, D.J. Saville, S. Wanwimolruk, Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds, J. Pharm. Pharm. Sci. 4 (2001) 217–227.

[70] V. Burkina, V. Zlabek, R. Halsne, E. Ropstad, G. Zamaratskaia, In vitro effects of the citrus flavonoids diosmin, naringenin and naringin on the hepatic drug-metabolizing CYP3A enzyme in human, pig, mouse and fish, Biochem. Pharmacol. 110–111 (2016) 109–116, https://doi.org/10.1016/j.bcp.2016.04.011.
[71] X. Wang, A.W. Wolkoff, M.E. Morris, Flavonoids as a novel class of human organic anion-transporting polypeptide OATP1B1 (OATP-C) modulators, Drug Metab. Dispos. 33 (2005) 1666–1672, https://doi.org/10.1124/dmd.105.005926.
[72] T. Kawasaki, Y. Shiozaki, N. Nomura, K. Kawai, Y. Uwai, T. Nabekura, Investigation of fluorescent substrates and substrate-dependent interactions of a drug transporter organic anion transporting polypeptide 2B1 (OATP2B1, Pharm. Res. 37 (2020) 115, https://doi.org/10.1007/s11095-020-02831-x.
[73] H. Zhi, Y. Yuan, C. Zhang, Y. Jiang, H. Zhang, C. Wang, J. Ruan, Importance of OATP1B1 and 1B3 in the liver uptake of luteolin and its consequent glucuronidation metabolites, J. Agric. Food Chem. 68 (2020) 2063–2070, https://doi.org/10.1021/acs.jafc.9b06954.
[74] C. Deng, C. Gao, X. Tian, B. Chao, F. Wang, Y. Zhang, J. Zou, D. Liu, Pharmacokinetics, tissue distribution and excretion of luteolin and its major metabolites in rats: Metabolites predominate in blood, tissues and are mainly excreted via bile, J. Funct. Foods 35 (2017) 332–340, https://doi.org/10.1016/j.jff.2017.05.056.
[75] Z. Wang, M. Zeng, Z. Wang, F. Qin, J. Chen, Z. He, Dietary luteolin: a narrative review focusing on its pharmacokinetic properties and effects on glycolipid metabolism, J. Agric. Food Chem. 69 (2021) 1441–1454, https://doi.org/10.1021/acs.jafc.0c08085.
[76] T. Morita, T. Akiyoshi, R. Sato, Y. Uekusa, K. Katayama, K. Yajima, A. Imaoka, Y. Sugimoto, F. Kiuchi, H. Ohtani, Citrus fruit-derived flavanone glycoside narirutin is a novel potent inhibitor of organic anion-transporting polypeptides, J. Agric. Food Chem. 68 (2020) 14182–14191, https://doi.org/10.1021/acs.jafc.0c06132.
[77] Y. Shirasaka, M. Shichiri, T. Mori, T. Nakanishi, I. Tamai, Major active components in grapefruit, orange, and apple juices responsible for OATP2B1-mediated drug interactions, J. Pharm. Sci. 102 (2013) 280–288, https://doi.org/10.1002/jps.23357.
[78] M.N. Chabane, A.A. Ahmad, J. Peluso, C.D. Muller, G. Ubeaud-Séquier, Quercetin and naringenin transport across human intestinal Caco-2 cells, J. Pharm. Pharmacol. 61 (2009) 1473–1483, https://doi.org/10.1211/jpp.61.11.0006.
[79] N. Orrego-Lagarón, M. Martínez-Huélamo, A. Vallverdú-Queralt, R.M. Lamuela-Raventos, E. Escribano-Ferrer, High gastrointestinal permeability and local metabolism of naringenin: influence of antibiotic treatment on absorption and metabolism, Br. J. Nutr. 114 (2015) 169–180, https://doi.org/10.1017/S0007114515001671.
[80] N. Orrego-Lagarón, M. Martínez-Huélamo, P. Quifer-Rada, R.M. Lamuela-Raventos, E. Escribano-Ferrer, Absorption and disposition of naringenin and quercetin after simultaneous administration via intestinal perfusion in mice, Food Funct. 7 (2016) 3880–3889, https://doi.org/10.1039/C6FO00633G.
[81] T. Isobe, S. Ohkawara, S. Ochi, T. Tanaka-Kagawa, H. Jinno, N. Hanioka, Naringenin glucuronidation in liver and intestine microsomes of humans, monkeys, rats, and mice, Food Chem. Toxicol. 111 (2018) 417–422, https://doi.org/10.1016/j.fct.2017.11.057.