Reversible Inhibition of Human Carboxylesterases by Acyl Glucuronides

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Running Title:
Reversible Inhibition of hCESs by Acyl Glucuronides

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Abbreviations:
4NP – para-nitrophenol
4NPA – para-nitrophenyl acetate
CES – carboxylesterase
CGP 47292 – (2S,3S,4S,5R,6S)-6-((1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carbonyl)oxy)-3,4,5-
trihydroxytetrahydro-2H-pyran-2-carboxylic acid
hCES – human carboxylesterase
ABSTRACT
Carboxylesterases hydrolyze esters, amides, and thioesters to produce carboxylic acids and resulting alcohols, amines, and thiols, respectively. UDP-glucuronosyltransferases are co-localized with carboxylesterases and have the potential to further metabolize carboxylic acids to acyl glucuronides, but it is currently unknown if acyl glucuronides, being esters, also interact with carboxylesterases. **OBJECTIVE:** This study explores the ability of acyl glucuronides to act as substrates or inhibitors of human carboxylesterases 1 (hCES1) and 2 (hCES2).

**METHODS:** The stability of six acyl glucuronides in the presence of hCES1, hCES2, and buffer alone (100 mM potassium phosphate, pH 7.4, 37 °C) were investigated. Reversible inhibition of 4-nitrophenyl acetate hydrolysis by the acyl glucuronides was also studied. Diclofenac-β-D-glucuronide was used to explore potential time-dependent inactivation. **RESULTS:** The chemical stability half-life values for CGP 47292-β-D-glucuronide, diclofenac-β-D-glucuronide, (R)-naproxen-β-D-glucuronide, (S)-naproxen-β-D-glucuronide, ibuprofen-β-D-glucuronide (racemic), clopidogrel-β-D-glucuronide, and valproate-β-D-glucuronide were found to be 0.252, 0.537, 0.996, 1.77, 3.67, 5.02, and 15.2 hours, respectively. Diclofenac-β-D-glucuronide, clopidogrel-β-D-glucuronide, ibuprofen-β-D-glucuronide, (R)-naproxen-β-D-glucuronide, and (S)-naproxen-β-D-glucuronide selectively inhibited hCES1 with Ki values of 4.32 ± 0.47, 24.8 ± 4.2, 355 ± 38, 468 ± 21, 707 ± 64 µM, respectively, but did not significantly inhibit hCES2. Valproate-β-D-glucuronide and CGP 47292-β-D-glucuronide did not inhibit either hCES. Time-dependent inactivation of hCES1 by diclofenac-β-D-glucuronide was not observed. Lastly, both hCES1 and hCES2 were shown not to catalyze the hydrolysis of the acyl glucuronides studied. **CONCLUSION:** Drug-drug interaction studies may be warranted for drugs that metabolize to acyl glucuronides due to the potential inhibition of hCESs.
INTRODUCTION

While there are five subfamilies of carboxylesterases (CESs) in humans (Williams et al., 2010), human carboxylesterase 1 (hCES1) and human carboxylesterase 2 (hCES2) have been well studied in xenobiotic metabolism for their high expression in the liver and intestine, respectively (Redinbo and Potter, 2005). CESs hydrolyze esters, amides, or thioesters to produce carboxylic acids and the corresponding alcohols, amines, or thiols. The electrophilic characteristics of the carbonyl carbon predispose a compound to bind CESs (Satoh and Hosokawa, 2006).

Acyl glucuronides are formed as the result of phase II metabolism by UDP-glucuronosyltransferases (UGTs) where carboxylic acids are bound to a glucuronic acid via an ester bond. Although considered a detoxification reaction, the reactivity of acyl glucuronides has been suggested to cause the toxicity of some drugs containing carboxylic acids (Regan et al., 2010). Some carboxylic acid drugs that form reactive acyl glucuronide metabolites, such as zomepirac and benoxaprofen, have been removed from the market due to their toxicity (Regan et al., 2010). The reactivity of these acyl glucuronides comes from the electrophilic characteristics of the ester carbon and its ability to attract a nucleophile and undergo nucleophilic substitution reactions (Shipkova et al., 2003).

Both CESs and UGTs are localized in the lumen of the endoplasmic reticulum (Satoh and Hosokawa, 2006). Being a CES metabolite, the potential exists for the carboxylic acid to be further metabolized by a UGT, forming an acyl glucuronide. However, the impact of this acyl glucuronide on the CESs is currently unknown and could result in a drug-drug interaction, especially since the two enzyme systems could be spatially close. This study was conducted to determine the potential of the CESs to recognize the acyl glucuronides as substrates or inhibitors.

MATERIALS AND METHODS

Sources of Materials:

4-Nitrophenol (4NP) and 4-nitrophenyl acetate (4NPA) were both obtained from Sigma-Aldrich Corp. (St. Louis, MO). The enzyme systems, human carboxylesterase 1 (hCES1) supersomes, human carboxylesterase 2 (hCES2) supersomes, and insect control supersomes, were obtained from BD Biosciences (Woburn, MA) and have been previously characterized (Wang et al., 2011). Phosphate buffer was also obtained from BD Biosciences (Woburn, MA). The acyl glucuronides, valproate-β-D-glucuronide, ibuprofen-β-D-glucuronide, clopidogrel-β-D-glucuronide, (R)-naproxen-β-D-glucuronide, (S)-naproxen-β-D-glucuronide, and diclofenac-β-D-glucuronide, were obtained from Toronto Research Chemicals Inc. (North York, Ontario, Canada). All glucuronide stock solutions and any subsequent dilutions were made using dimethyl sulfoxide (DMSO) as the solvent. The structures of the acyl glucuronides are given in Figure 1.

Synthesis of CGP 47292-β-D-glucuronide:

Synthesis of (2S,3R,4S,5S,6S)-6-((benzyloxy)carbonyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl 1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxylate: Preparation la: N-methylmorpholine (39 µL, 0.355 mmol) was added to a stirred solution of O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HATU; 67.6 mg, 0.178 mmol), 1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxylic acid (44.5 mg, 0.186 mmol; Ajinomoto Omnichem; Balen, Belgium) and benzyl D-glucuronate (50.0 mg, 0.176 mmol; Carbosynth Ltd.;
Compton, UK) in acetonitrile (1.8 mL) at room temperature under nitrogen. After approximately 26 hours at room temperature the mixture was evaporated. The residue was diluted with dichloromethane (DCM) and purified by chromatography on silica gel eluting with DCM containing methanol (0 - 30%) to yield the title compound, 53.8 mg (60%), as white foam (following addition and evaporation of acetone). The product was re-purified by chromatography on silica gel with DCM containing ethanol (5 - 10%) to give two components, title compound which had residual impurities (12.7 mg) and pure title compound (23.7 mg, 27%).

Preparation 1b: N-methylmorpholine (230 µL, 2.09 mmol) was added to 1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxylic acid (250.9 mg, 1.05 mmol; Ajinomoto Omnichem) in acetonitrile (9 mL) at room temperature. To this solution was added HATU (396.6 mg, 1.05 mmol), and benzyl D-glucuronate (297.0 mg, 1.045 mmol; Carbosynth Ltd.). The mixture was stirred at room temperature under a nitrogen atmosphere. All solvent had evaporated when checked after 3.25 hours therefore further acetonitrile (9 mL) was added and stirring continued at room temperature. After 5 hours the mixture was concentrated and the residue purified by chromatography on silica gel with DCM containing methanol (0 - 10%) to give two batches of the title compound, batch 1 (221.9 mg, 42%) as a pink foam and batch 2 (with slightly faster running impurity, 81.7 mg, 16%). Total yield was 303.6 mg, 58%. Batch 1 was re-purified by chromatography on silica gel with DCM containing methanol (0 - 10%) as eluent, to give the title compound, 192.1 mg (36%) as a white solid. Batch 2 was re-purified by chromatography on silica gel with DCM containing methanol (0 - 10%) to give the title compound as a white solid (47.0 mg, 9%). Total yield was 239.1 mg (45%).

Synthesis of (2S,3S,4S,5R,6S)-6-((1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carbonyl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (CGP 47292-β-D-glucuronide): (2S,3R,4S,5R,6S)-6-((benzyloxy)carbonyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl 1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxylate (45.4 mg, 0.09 mmol), propan-2-ol (1.0 mL) and tetrahydrofuran (1.0 mL) were stirred with 10% palladium on carbon (10.0 mg) under a hydrogen atmosphere for 1 hour. The mixture was diluted with tetrahydrofuran:propan-2-ol (1:1), total volume used was 30 mL. The filtrate was concentrated. DCM was added (white precipitate formed) and evaporated to leave an opaque oil. Water (20 mL) was added and the mixture warmed (not transparent). DCM (20 mL) was added, the mixture shaken then filtered through a hydrophobic frit. The aqueous layer was shaken further with DCM (20 mL) and re-filtered through a hydrophobic frit. The aqueous phase was evaporated to give a white solid that was dried under vacuum overnight to give the title compound (32.8 mg, 88%), as a white solid.

Acyl Glucuronide Chemical Stability:

The final glucuronide concentration for each reaction was 10 µM with 100 mM phosphate buffer at pH 7.4 and 1% DMSO. Each reaction was run in duplicate at 37 °C. These reactions were stopped at 0, 0.5, 1, 2, 4, and 6 hours for all compounds except for diclofenac-β-D-glucuronide, which was stopped at 0, 10, 30, 60, 90, and 120 minutes, using an internal standard(IS)/quench solution containing both probenecid and rose bengal, which also were utilized as calibrants for the MS-TOF. This IS/quench solution contained 10% acetic acid for all acyl glucuronide solutions except for clopidogrel-β-D-glucuronide, which contained only 0.5% acetic acid. The IS/quench solution for clopidogrel-β-D-glucuronide was used to keep the samples near a pH of 7 since this acyl glucuronide was shown to be less stable at a more acidic or basic pH (Silvestro et al., 2011). Samples were analyzed...
using LC/MS-TOF. Linear regression of the natural log of the peak areas ratio versus time was used to calculate the half-life values, assuming pseudo-first order kinetics.

**Acyl Glucuronide Enzyme Stability:**

The maximum acyl glucuronide concentration was 100 µM with a final DMSO concentration of 1%. The CESs have been previously shown to tolerate DMSO up to a final concentration of 2% (Williams et al., 2008). The enzyme-containing solutions (insect control, hCES1, and hCES2) were prepared to a final protein concentration of 10 µg/mL in 100 mM phosphate buffer at pH 7.4 and pre-warmed to 37 °C. Reactions were initiated by the addition of the 1:1 serially-diluted acyl glucuronide solution and terminated at 0, 10, 30, and 60 minutes using the same IS/quench solutions stated in the previous section. Samples were analyzed using LC/MS-TOF.

**Analysis by LC/MS-TOF**

Samples were injected onto an AB Sciex QSTAR Elite mass spectrometer (Applied Biosystems; Toronto, Canada). The MS-TOF contained a TurboIonSpray interface, which operated in negative mode at a temperature of 500 °C. Its ionspray voltage was set to -4200 V, while the curtain, nebulizing, and desolvation gas settings were 25, 40, and 75, respectively. The declustering, focusing, and declustering-2 potentials were -10, -180, and -5, respectively. The liquid chromatography system contained Shimadzu LC-20AD pumps (Kyoto, Japan) along with an SIL-HTc autosampler. Chromatographic separation of the acyl glucuronides along with their respective metabolites used a Synergi 4µ Fusion RP80A 150 × 2.00 mm chromatography column (Phenomenex; Torrance, CA) at room temperature, 5 mM ammonium acetate (mobile phase A), and acetonitrile (mobile phase B), which was directed to the MS-TOF from 2.0 to 55.0 minutes. Long gradient profiles were implemented to separate the 1-O-acyl glucuronides from the migrated acyl glucuronides. The gradient profile changed from 1% B at 0.00 minutes to 20% B at 30.00 minutes, from 20% B at 35.00 minutes to 99% B at 50.00 minutes, and 99% B at 54.00 minutes to 1% B at 54.10 minutes with a total run time of 60.00 minutes. This gradient profile was for all acyl glucuronides tested except for CGP 47292-β-D-glucuronide, clopidogrel-β-D-glucuronide, and valproate-β-D-glucuronide. The gradient profile for CGP 47292-β-D-glucuronide was changed from 1% B at 0.00 minutes to 20% B at 45.00 minutes to 99% B at 50.00 minutes and 99% B at 54.00 minutes to 1% B at 54.10 minutes with a total run time of 60.00 minutes. The gradient profile for clopidogrel-β-D-glucuronide was changed from 1% B at 0.00 minutes to 20% B at 30.00 minutes, 20% B at 35.00 minutes to 99% B at 40.00 minutes and then to 1% B at 40.01 minutes with a total run time of 45.00 minutes. The gradient profile for valproate-β-D-glucuronide was changed from 1% B at 0.00 minutes to 35% B at 30.00 minutes, from 35% B at 33.00 minutes to 99% B at 50.00 minutes, and from 99% B at 54.00 minutes to 1% B at 54.10 minutes with a total run time of 60.00 minutes. Sample analysis was conducted using Analyst QS 2.0 (Applied Biosystems; Foster City, CA), and the peak areas of the acyl glucuronides were normalized to the peak areas for probenecid. Peak areas were determined by a window of 0.01 Da around the exact mass of each respective compound analyzed. Chromatograms for CGP 47292-β-D-glucuronide and clopidogrel-β-D-glucuronide are presented in Figure 2.

**Inhibition of CESs by Acyl Glucuronides:**

To determine if inhibition by the acyl glucuronides had occurred, the impact on the hydrolysis of 4NPA to 4NP was assessed. The maximum final concentration of the acyl glucuronides was 1 mM with 200 µM 4NPA and
1.5% DMSO. The enzyme-containing solutions (hCES1 and hCES2) were prepared to a final protein concentration of 10 µg/mL in 100 mM phosphate buffer at pH 7.4. The reactions were conducted in duplicate and monitored at an absorption value of 405 nm using a temperature-controlled spectrophotometer at 37 °C. Data from a standard curve, with a final maximum 4NP concentration of 100 µM, was also collected in a similar manner. Water was added to the surrounding wells of the plate to reduce the edge effect. Acyl glucuronides that demonstrated significant inhibition at 1 mM (data not shown) were further studied to produce a Kᵢ plot in triplicate using the same parameters mentioned above with the exception of a serially-diluted range of 4NP concentrations up to 1 mM instead of a single 4NP concentration of 200 µM.

The slope and y-intercept of the standard curve were calculated in GraphPad Prism 5 (La Jolla, CA), with appropriate weighting. Microsoft Excel (Redmond, WA) was used to calculate the amount of 4NP hydrolysis and the reaction rate. The reaction rate was exported to Prism 5 to determine the Kᵣ, Vₘₐₓ, and Kᵢ values.

Time-dependent inactivation of diclofenac-β-D-glucuronide was tested with a potential shift of the IC₅₀ curves produced from a pre-incubation of the acyl glucuronide with hCES1 at three time-points (10, 30, and 60 minutes) prior to the addition of 4NPA as the probe substrate. The final 4NPA concentration used in this assay was 550 µM, the approximate Kᵣ value determined when analyzing the Kᵢ values. The hCES1-containing solution was prepared to a final protein concentration of 10 µg/mL in 100 mM phosphate buffer at pH 7.4. All reactions were performed in triplicate. Data was collected and analyzed in a similar manner as described above.

RESULTS AND DISCUSSION

Acyl Glucuronide Chemical Stability

Acyl glucuronides are known to have varying degrees of chemical instability due to acyl migration (Regan et al., 2010). Therefore, the acyl glucuronide stability values were obtained to demonstrate sufficient stability to conduct the substrate and inhibition studies. Table 1 lists the half-life values obtained in this study as well as values obtained in previous studies. Each enantiomer was chromatographically separated from the racemic mixture of ibuprofen-β-D-glucuronide to produce half-life values of 1.29 and 2.25 hours that are consistent with the 1.8-hour half-life of (R)-ibuprofen-β-D-glucuronide and the 3.3-hour half-life of (S)-ibuprofen-β-D-glucuronide (Akira et al., 2000). Despite literature half-life values of 60 hours (Ethell et al., 2003) and 79 hours (Regan et al., 2010), valproate-β-D-glucuronide’s experimental half-life was found to be only 15.2 hours and similar to the results obtained internally when valproate-β-D-glucuronide was used as a control (data not shown). Since this study in addition to the previous studies using valproate-β-D-glucuronide have extrapolated the half-life values beyond the last time point tested, the data should be interpreted as qualitatively similar. Therefore, the data demonstrated the high chemical stability of valproate-β-D-glucuronide. Two of the compounds studied do not have published half-life values for chemical stability, CGP 47292-β-D-glucuronide and clopidogrel-β-D-glucuronide, and are first reported here. Overall, the stability was adequate to support the currently reported assessments as potential hCES substrates and inhibitors.

Enzyme Stability of Acyl Glucuronides

The slope and y-intercept of the standard curve were calculated in GraphPad Prism 5 (La Jolla, CA), with appropriate weighting. Microsoft Excel (Redmond, WA) was used to calculate the amount of 4NP hydrolysis and the reaction rate. The reaction rate was exported to Prism 5 to determine the Kᵣ, Vₘₐₓ, and Kᵢ values.
The loss of parent for all acyl glucuronides tested with both hCES1 and hCES2 did not exceed that of the insect control (data not shown). Therefore, it was concluded that the hCESs do not seem to significantly hydrolyze these compounds, if at all, under the current experimental conditions.

Reversible Inhibition of CESs by Acyl Glucuronides

Most acyl glucuronides tested in this study showed selective inhibition for hCES1 over hCES2 up to a top concentration of 1 mM (Table 1, Figure 3). Inhibition values could not be determined for neither CGP 47292-β-D-glucuronide and valproate-β-D-glucuronide with hCES1 nor for any compound with hCES2 with a top substrate concentration of 1 mM.

Table 1 reports the $K_i$ values for all acyl glucuronides in this study that showed inhibition for hCES1. The $K_m$ and $V_{max}$ values of 537 ± 24 µM and 3.19 ± 0.20 µmol/min/mg, respectively, for 4NPA hydrolysis remained consistent for the compounds tested. It is interesting to note that the two most inhibitory acyl glucuronides were diclofenac-β-D-glucuronide and clopidogrel-β-D-glucuronide, which both contain chlorophenyl moieties. There also appeared to be a loose correlation between inhibitory potency ($K_i$ value) and some chemical properties of these acyl glucuronides, such as ClogP. As the inhibition strength of the acyl glucuronides tested increased, the value for ClogP increased (Table 1). Increased inhibition with increased ClogP values has been previously reported (Wadkins et al., 2007).

The aglycones for 2 of the acyl glucuronides tested have been previously assessed for their inhibition of hCES1 and hCES2 (Williams et al., 2011). Valproate selectively inhibited hCES1 over hCES2 with $K_i$ values of 363 µM and 7.9 mM, respectively, but neither hCES was inhibited by CGP 47292 up to a concentration of 1 mM. Similar to valproate-β-D-glucuronide, valproyl-CoA had reduced hCES inhibition compared to valproate. No definitive conclusion can be drawn at this time concerning the inhibitory differences between the acyl glucuronides and their respective aglycones.

Time-Dependent Inactivation of hCES1 by Diclofenac-β-D-Glucuronide

Because of the relatively short half-life value of diclofenac-β-D-glucuronide and its reactivity to undergo both acyl migration and protein adduction, this experiment was designed to test this acyl glucuronide’s inhibition strength. The 10, 30, and 60 minute time points were chosen to determine inhibition potency with roughly 20%, 50%, and 75% loss of the 1-O-acyl glucuronide. Both diclofenac-β-D-glucuronide and hCES1 were co-incubated prior to the addition of 4NPA as the probe substrate. IC$_{50}$ values of 2.51 µM at 10 min, 1.96 µM at 30 min, and 2.27 µM at 60 min were found. Because of this similarity in inhibition potency, it was concluded that no time-dependent inactivation appears to have occurred.

Drug-Drug Interactions (DDI)

Currently, no report has shown a DDI between an acyl glucuronide and an hCES. A previous report indicated coadministration of rufinamide and valproate resulted in higher plasma levels of rufinamide in children (Perucca et al., 2008). The major metabolic pathway for rufinamide is hydrolysis, mainly hCES1, to CGP 47292 with further acyl glucuronidation. Valproate has two primary metabolic pathways, mitochondrial β-oxidation and glucuronidation (Argikar and Remmel, 2009). In an effort to better understand the potential drug-drug interaction (DDI), the impact of CGP 47292, valproate, and valproyl-CoA on hCES-mediated hydrolysis were assessed.
(Williams et al., 2011), but none of the compounds showed substantial inhibition of hCES-mediated hydrolysis of rufinamide. Based on the results of the current study, CGP 47292-β-D-glucuronide and valproate-β-D-glucuronide are not likely to cause the observed in vivo DDI.

Interestingly, diclofenac-β-D-glucuronide showed potent reversible inhibition of hCES1 with a $K_i$ value of 4.32 µM. Diclofenac is estimated to undergo glucuronidation 3-fold faster than hydroxylation (Kumar et al., 2002), indicating the primary metabolite to be the acyl glucuronide. Therefore, co-administration with a compound that is mainly metabolized by hCES1 with a $K_m$ value greater than 5 µM could result in a potential DDI, including clopidogrel (Tang et al., 2006), methylphenidate (Sun et al., 2004), and oseltamivir (Shi et al., 2006).

**Conclusion**

With the exception of valproate-β-D-glucuronide, all the acyl glucuronides tested in this study showed selective inhibition of hCES1 over hCES2. The hydrolysis of the acyl glucuronides by both hCES1 and hCES2 did not exceed that of the insect control, indicating the CESs did not significantly hydrolyze these compounds, if at all. However, inhibition by many of the acyl glucuronides was observed suggesting acyl glucuronides may be more potent inhibitors of hCES1 than substrates. Lastly, in testing diclofenac-β-D-glucuronide as a potential time-dependent inactivator, the 10, 30, and 60 minute pre-incubations demonstrated similar IC$_{50}$ values suggesting diclofenac-β-D-glucuronide does not appear to be a time-dependent inactivator of hCES1. Therefore, drug-drug interaction studies may be warranted for drugs that form acyl glucuronides due to their potential to inhibit human carboxylesterases.
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AUTHORSHIP CONTRIBUTIONS
Participated in research design: Hall, Lai, Williams
Conducted experiments: Inoue, Hall, Williams
Contributed new reagents or analytical tools: Hall
Performed data analysis: Inoue, Williams
Wrote or contributed to the writing of the manuscript: Inoue, Hall, Lai, Williams
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FIGURE LEGEND

Figure 1 – Chemical structures for all acyl glucuronides in this study.

Figure 2 – Chromatograms showing separation of clopidogrel-β-D-glucuronide and CGP 47292-β-D-glucuronide from their respective acyl migrants. The chromatogram for CGP 47292-β-D-glucuronide suggests the separation of the 4-, 3-, 1-, and 2-O-β-glucuronides, respectively, based on the time-dependent formation.

Figure 3 – Ki graphs for the acyl glucuronides that showed inhibition of hCES1.
Table 1 – Acyl Glucuronide chemical stability and reversible inhibition of hCESs. Compounds are listed in increasing order by their chemical stability. The ClogP values were determined using ChemBioDraw.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ClogP</th>
<th>Chemical Stability</th>
<th>Ki (µM) with hCES1</th>
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<tr>
<td></td>
<td></td>
<td>t_{1/2} (hours)</td>
<td></td>
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<tr>
<td>CGP 47292-β-D-Glucuronide</td>
<td>-0.753</td>
<td>0.252</td>
<td>NA</td>
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<tr>
<td>Diclofenac-β-D-Glucuronide</td>
<td>2.92</td>
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<td>1.01</td>
<td>0.996</td>
<td>0.92^a</td>
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<td>(S)-Naproxen-β-D-Glucuronide</td>
<td>1.01</td>
<td>1.77</td>
<td>1.8^a</td>
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<td>Ibuprofen-β-D-Glucuronide</td>
<td>1.87</td>
<td>3.67 (1.29, 2.25)^*</td>
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<td></td>
<td></td>
<td>racemic=3.5^b</td>
<td>(R)=1.8^c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S)=3.3^c</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>355 ± 38</td>
<td></td>
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<tr>
<td>Clopidogrel-β-D-Glucuronide</td>
<td>1.99</td>
<td>5.02</td>
<td>NA</td>
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<tr>
<td>Valproate-β-D-Glucuronide</td>
<td>0.953</td>
<td>15.2</td>
<td>60^d, 79^a</td>
</tr>
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</table>

NA – not available
NT – not tested due to minor inhibition at 1 mM
^ These 2 values were determined by chromatographic separation of the racemic mixture, but were not identified as the (R) or (S) enantiomer.

a: (Regan et al., 2010); b: (Vanderhoeven et al., 2006); c: (Akira et al., 2000); d: (Ethell et al., 2003)
Figure 1

Clopidogrel-β-D-Glucuronide

(R)-Naproxen-β-D-Glucuronide

(S)-Naproxen-β-D-Glucuronide

Ibuprofen-β-D-Glucuronide

Valproate-β-D-Glucuronide

CGP 47292-β-D-Glucuronide

Gluc =