

# Effects of $\beta$ -Blockers and Tricyclic Antidepressants on the Activity of Human Organic Anion Transporting Polypeptide 1A2 (OATP1A2)<sup>§</sup>

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## ABSTRACT

The organic anion transporting polypeptide 1A2 (OATP1A2), a membrane drug transporter expressed on important organs (such as the brain, kidney, and intestine) may be a key element in the disposition of drugs. Previous studies demonstrated that it could transport a broad spectrum of substrates, including endogenous molecules and clinically relevant drugs, such as several  $\beta$ -blockers and 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors. The primary objective of this study was to investigate OATP1A2 transport activity using rosuvastatin as a probe substrate and evaluate competitive inhibition of its transport by  $\beta$ -blockers. Rosuvastatin transport was saturable, with a  $K_m$  of 60.2  $\mu$ M. With the exception of carvedilol ( $IC_{50}$  of 3.2  $\mu$ M), all of

the other  $\beta$ -blockers that were evaluated had a small or insignificant effect on OATP1A2-mediated uptake of rosuvastatin. Carvedilol differs from the other  $\beta$ -blockers by the tricyclic moiety in its chemical structure. As a secondary objective, the transport of a series of tricyclic compounds by OATP1A2 and their potential for rosuvastatin transport inhibition were evaluated. Tricyclic compounds were not OATP1A2 substrates. On the other hand, tricyclic compounds with a short aliphatic amine chain inhibited OATP1A2-mediated rosuvastatin transport. Our data suggest that these drugs may modulate the transport of OATP1A2 substrates and may affect drug actions.

## Introduction

Organic anion transporting polypeptide 1A2 (OATP1A2) is a membrane drug transporter expressed on organs important for drug disposition. The mRNA expression is found to be the highest in the brain, followed by the kidney, liver, lung, and testes (Kullak-Ublick et al., 1995). OATP1A2 protein is found on the luminal membrane of the brain capillary endothelial cells, which make up the blood-brain barrier, apical membranes of cholangiocytes in the liver, apical membrane of the distal nephrons in the kidney, and apical membrane of enterocytes in the duodenum (Gao et al., 2000; Lee et al., 2005; Glaeser et al., 2007). OATP1A2 expression in the small intestine is controversial, as recent studies could not detect its presence in the entire intestine (Groer et al., 2013; Drozdik et al., 2014). Due to its location, the following roles have been attributed to OATP1A2: distribution of substrates to the brain, reabsorption of substrates

excreted in the bile, reabsorption or secretion of xenobiotics into urine, and oral absorption of xenobiotics.

OATP1A2 transports various endogenous molecules, such as bile salts and hormones (triiodothyronine, thyroxine, and steroid conjugates) (Kullak-Ublick et al., 1995, 1998; Fujiwara et al., 2001; Lee et al., 2005). Based on the endogenous substrates it transports and its localization, it has been proposed that OATP1A2 may be involved in the regulation of several physiologic processes. For instance, it may be implicated in the delivery of thyroid hormones to the brain and kidney as well as the removal of thyroid hormones from the periphery (Hagenbuch, 2007). In addition, it may play a role in bile acid transport, as a study has shown that OATP1A2 mRNA is upregulated in patients with cholestatic liver disease (Kullak-Ublick et al., 1997).

OATP1A2 can also transport several exogenous substances, including peptide agonists of the  $\sigma$ -opioid receptor and bromosulphophthalein (Kullak-Ublick et al., 1995; Gao et al., 2000). Several clinically important drugs, such as fexofenadine, imatinib, methotrexate, pravastatin, and rosuvastatin, are also transported by OATP1A2 (Cvetkovic et al., 1999; Badagnani et al., 2006; Ho et al., 2006; Hu et al., 2008; Shirasaka et al., 2010). Rosuvastatin is a hydrophilic molecule and, therefore, depends on transporters to move across the plasma membrane. Rosuvastatin has a high affinity for OATP transporters, as its

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**ABBREVIATIONS:** AUC, area under the plasma concentration time curve; CNS, central nervous system; HEK, human embryonic kidney; OATP, organic anion transporting polypeptide; PBS, phosphate-buffered saline.

$K_m$  was determined to be 2.6, 4.0, 9.8, and 2.4  $\mu\text{M}$  for OATP1A2, OATP1B1, OATP1B3, and OATP2B1, respectively (Ho et al., 2006). Finally, OATP1A2 is inhibited by various flavonoids, such as naringin, apigenin, kaempferol, quercetin, and several flavonoids found in green tea (Bailey et al., 2007; Mandery et al., 2010; Roth et al., 2011; Misaka et al., 2014).

Flavonoids are found in vegetables, fruits, and plants; thus, pharmacokinetic studies using flavonoids focused on intestinal interactions. Bailey et al. (2007) have demonstrated that ingestion of fexofenadine and a solution of naringin decreased fexofenadine maximum plasma concentration ( $C_{\text{max}}$ ) and the area under the plasma concentration time curve (AUC). Misaka et al. (2014) have shown that green tea decreased nadolol  $C_{\text{max}}$  and AUC. These interactions assume that OATP1A2 is an intestinal uptake transporter in the human intestine.

Single-nucleotide polymorphisms in the gene encoding OATP1A2, *SLCO1A2*, resulting in impaired cell surface expression and reduced OATP1A2 activity have been discovered in healthy individuals (Lee et al., 2005; Badagnani et al., 2006; Laitinen and Niemi, 2011). This suggests that OATP1A2 may not play a fundamental role in physiologic functions but may act as a secondary transporter for endogenous molecules. However, there is evidence that OATP1A2 may be important in drug disposition, as Yamakawa et al. (2011) demonstrated that imatinib clearance is affected in chronic myeloid leukemia patients with the *SLCO1A2*-361G>A genotype.

A previous study has proposed that several  $\beta$ -blockers are OATP1A2 substrates (Kato et al., 2009). Initially, their study aimed at determining the transporters involved in the gastrointestinal absorption of celiprolol to understand the food-drug interaction induced by citrus juices using an animal model. They demonstrated an increase in plasma concentrations of celiprolol in *mdr1a/b*<sup>-/-</sup> mice compared with wild-type mice. Using isolated tissues of the mouse small intestine, they demonstrated competitive inhibition between celiprolol and bromosulphophthalein for transport from the apical to basal side. Their results suggested the involvement of P-glycoprotein and an influx transporter in the absorption of celiprolol. Subsequently, using *Xenopus laevis* oocytes, the uptake transporter involved was shown to be OATP1A2. In addition, they tested several other  $\beta$ -blockers and showed that acebutolol, atenolol, nadolol, sotalol, and labetalol are OATP1A2 substrates. More recently, a study has also shown that nadolol is transported by OATP1A2 in human embryonic kidney (HEK293) cells stably expressing the transporter (Misaka et al., 2014).

Considering the OATP1A2 location on important organs involved in drug disposition (such as the brain), it is of interest to investigate this transporter further. Based on current knowledge, the primary objectives of this study were to 1) assess rosuvastatin as a probe substrate for OATP1A2; and 2) determine whether there is competition between rosuvastatin and  $\beta$ -blockers for transport through OATP1A2. Considering the results obtained throughout the course of our studies, the secondary objectives were to 1) evaluate the transport of different tricyclic compounds through OATP1A2; and 2) determine whether there is competition between rosuvastatin and tricyclic compounds for transport through OATP1A2. Experiments were conducted using a HEK293 cell line stably overexpressing OATP1A2. This *in vitro* model was used since it offers many advantages over transient models, such as stable expression, possibility for high-throughput screening, and ease of use once the cell line has been established.

## Materials and Methods

Acebutolol hydrochloride, alprenolol tartrate salt, amitriptyline hydrochloride, atenolol, carbamazepine, carbazole, chlorpromazine hydrochloride, clomipramine hydrochloride, desipramine hydrochloride, imipramine hydrochloride, metoprolol tartrate salt, nadolol, naproxen, nortriptyline hydrochloride, phenothiazine, propranolol hydrochloride, timolol maleate salt, and trimipramine maleate salt were purchased from Sigma-Aldrich (St. Louis, MO). Carazolol hydrochloride, carvedilol, celiprolol hydrochloride, doxepin hydrochloride, and rosuvastatin calcium salt were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Sotalol hydrochloride was a gift from Bristol-Myers Squibb (Montreal, QC, Canada). All chemicals and solvents were obtained from Sigma-Aldrich, Fisher Scientific (Fair Lawn, NJ), or J.T. Baker (Center Valley, PA).

**Cell Culture.** HEK293-OATP1A2 and HEK293-VC cells were kindly provided by Dr. Markus Keiser and Dr. Werner Siegmund (Department of Clinical Pharmacology, Center of Drug Absorption and Transport, University Medicine Greifswald, Greifswald, Germany). The cells were cultured in minimum essential medium containing 10% fetal bovine serum, 1 $\times$  nonessential amino acids, and 1 $\times$  sodium pyruvate at 37°C and 5% CO<sub>2</sub>. Cell culture media and supplements were purchased from Multicell Wisent Inc. (St.-Jean-Baptiste, QC, Canada), whereas fetal bovine serum was obtained from HyClone Thermo Scientific (Logan, UT).

**Uptake Assays and Competition Assays.** HEK293-OATP1A2 and HEK293-VC cells were seeded in tissue culture plates (6 or 12 well) previously treated with poly-L-lysine (Sigma-Aldrich). The number of cells seeded in 6- and 12-well plates was 1.5  $\times$  10<sup>6</sup> and 7.5  $\times$  10<sup>5</sup> cells/well, respectively. After 24 hours, the culture media was removed and the cells were preincubated with a warm transport buffer (142 mM NaCl, 5 mM KCl, 1 mM K<sub>2</sub>HPO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 1.5 mM CaCl<sub>2</sub>, 5 mM glucose, and 12.5 mM HEPES, pH 7.3) at 37°C for 5 minutes. Following the preincubation period, the cells were incubated with a transport buffer containing rosuvastatin in the presence or absence of an inhibitor at 37°C for 2 minutes. After incubation, the cells were washed twice with phosphate-buffered saline (PBS) containing 10% acetonitrile followed by a final wash with PBS. Rosuvastatin transport (60  $\mu\text{M}$ ) at different time points was done in 6-well plates by incubating HEK293-OATP1A2 and HEK293-VC cells. The  $K_m$  and  $V_{\text{max}}$  of rosuvastatin transport through OATP1A2 was determined by incubating HEK293-OATP1A2 and HEK293-VC cells in 6-well plates with rosuvastatin at concentrations ranging from 10 to 250  $\mu\text{M}$ . To determine whether a compound can block OATP1A2, HEK293-OATP1A2 and HEK293-VC cells were coinubated in 12-well plates with rosuvastatin (150  $\mu\text{M}$ ), which was used as the probe substrate, in the absence or presence of different  $\beta$ -blockers (1.5–100  $\mu\text{M}$ ) or different tricyclic compounds (12.5 nM–250  $\mu\text{M}$ ). A concentration of 150  $\mu\text{M}$  rosuvastatin (three times the  $K_m$  value) was selected to saturate the OATP1A2 transporter with the probe substrate. The inhibitory constant ( $K_i$ ) of the tricyclic drugs for OATP1A2 was determined by incubating HEK293-OATP1A2 and HEK293-VC cells in 6-well plates with various concentrations of rosuvastatin (25–250  $\mu\text{M}$ ) in the absence or presence of the tricyclic drugs (0.5–50  $\mu\text{M}$ ). Uptake of carvedilol was assessed in 12-well plates at a concentration of 2  $\mu\text{M}$  for 2 minutes at 37°C. The preincubation and washing steps are the same as for rosuvastatin.

The protein concentration was measured in three wells of cells lysed with 1% SDS + 0.2 N NaOH using the Pierce BCA protein assay kit from Thermo Scientific (Rockford, IL).

**Quantification of Rosuvastatin by High-Performance Liquid Chromatography-UV.** The quantity of rosuvastatin transported in the cells was measured by high-performance liquid chromatography with UV detection. The instrumentation consisted of a SpectraSystem P4000 pump, SpectraSystem AS3000 autosampler, Finnigan SpectraSystem UV6000 UV detector, and SpectraSystem SN4000 system controller from Thermo Electron Corporation (San Jose, CA). ChromQuest version 4.2.34 software was used for data acquisition (Thermo

Electron Corporation). The samples were separated on a Phenomenex Luna 5u C8 column (150 × 4.6 mm, 5 μM; Phenomenex, Torrance, CA). The mobile phase consisted of a mixture containing 10 mM ammonium formate, pH 3, and acetonitrile (57:43 v/v). The flow rate was set at 1.2 ml/min, and the column was heated at 40°C. Naproxen was used as the internal standard. The retention times of rosuvastatin and naproxen were 4.8 and 6.1 minutes, respectively. The peaks were monitored at a wavelength of 243 nm. The lowest limit of quantification was 25 ng/ml. The calibration curve was linear between 25 and 25,000 ng/ml ( $r^2$  of 0.996). The interday coefficient of variation (CV) for the calibration curve using four levels of quality controls (25, 100, 2500, and 25,000 ng/ml) ranged between 3.9 and 7.8%. The interday accuracy ranged between 94.4 and 98.4%. The intraday CV for the four levels of quality control ranged between 1.1 and 13.6%. The intraday accuracy ranged between 84.5 and 101.4%.

After the final wash with PBS, the samples were processed as follows. The cells were lysed with methanol containing naproxen (100 ng/ml). The cell lysate was transferred to a 1.7-ml microtube, and the samples were spun down at a maximum speed for 10 minutes at room temperature. The supernatant was transferred to a culture borosilicate glass tube, evaporated to dryness, and reconstituted in 100 μl of 10 mM ammonium formate, pH 3, and acetonitrile (57:43 v/v). A volume of 20 μl per sample was injected.

**Quantification of Carvedilol by High-Performance Liquid Chromatography Fluorescence.** The same system was used for the quantification of carvedilol than for rosuvastatin, except for the detector, which was a SpectraSystem FL3000 fluorescence detector from Thermo Electron Corporation. The samples were separated on an Eclipse XDB-C8 column (150 × 4.6 mm, 5 μM; Agilent Technologies, Santa Clara, CA). The mobile phase consisted of a mixture of 50 mM potassium phosphate monobasic, pH 3.5, and acetonitrile (60:40 v/v). The flow rate was set at 1.0 ml/min, and the column was heated at 50°C. Propranolol was used as the internal standard. The retention times of carvedilol and propranolol were 2.9 and 2.2 minutes, respectively. The excitation and emission wavelength were 242 and 344 nm, respectively. After the final wash in PBS, the samples were processed similarly to the rosuvastatin samples, with the following exceptions: the cells were lysed with methanol containing propranolol (200 ng/ml), and the samples were reconstituted in 500 μl of 50 mM potassium phosphate monobasic, pH 3.5. The lowest limit of quantification was 50 ng/ml. The calibration curve was linear over a concentration range of 50 to 2000 ng/ml ( $r^2$  of 0.998). The interday CV for the calibration curve using four levels of quality controls (50, 80, 200, and 2000 ng/ml) ranged between 3.9 and 8.2%. The interday accuracy ranged between 95.3 and 99.3%. The intraday CV for the four levels of quality control ranged between 3.0 and 10.3%. The intraday accuracy ranged between 91.4 and 103.3%.

**Data Analysis.** The net transport of rosuvastatin through OATP1A2 was calculated by subtracting the value in the VC cells from the value in the OATP1A2 cells. Data were analyzed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Each data point is expressed as the mean ± S.D.  $K_m$  and  $V_{max}$  were calculated by fitting the data to the Michaelis-Menten equation.  $IC_{50}$  values were calculated by fitting the data to the log(inhibitor) versus response equation, and the range given represents the 95% confidence interval. Transport inhibitions of different concentrations of rosuvastatin by increasing concentrations of inhibitors were plotted in a Dixon plot. Linear regression was used to fit each set of data, and the intercept of all lines represents the  $-K_i$ . The  $K_i$  was accurately calculated by the equations

$$y = V_{max} \times \left( \frac{x}{K_{mapp} + x} \right)$$

$$K_{mapp} = K_m \times \left( 1 + \frac{l}{K_i} \right).$$

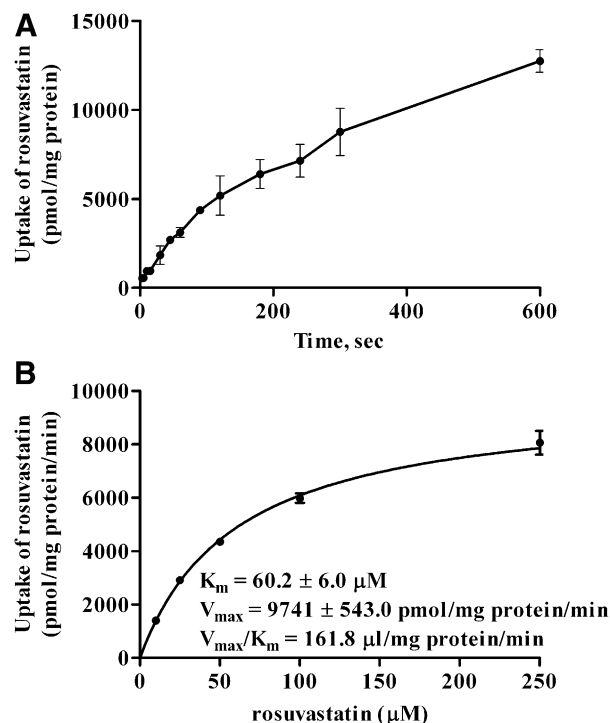
An initial value of 1.0 was set for all parameters. The rule for the initial value for  $K_m$  was set to \*XMID, and  $V_{max}$  was set to \*YMAX. A

shared value for all data sets was set as a default constraint for  $K_m$ ,  $K_i$ , and  $V_{max}$ , and a constant data set (= column title) was set for the parameter  $l$ .

## Results

**Transport of Rosuvastatin through OATP1A2.** Rosuvastatin transport via OATP1A2 was characterized using a HEK293 cell model stably expressing this transporter. Transport during different time points demonstrated that rosuvastatin uptake was linear between 1 and 2 minutes (Fig. 1A). An incubation time of 2 minutes was chosen for all experiments, as it remains in the linear range. Overall, the transport activity varied from 1395 to 8056 pmol/mg protein per minute over a range of concentrations of 10–250 μM of rosuvastatin. A saturable transport was observed with a  $K_m$  of  $60.2 \pm 6.0$  μM, the  $V_{max}$  was  $9741 \pm 543$  pmol/mg protein per minute, and the intrinsic clearance was  $161.8$  μl/mg protein per minute (Fig. 1B).

**Effect of β-Blockers on Rosuvastatin Uptake through OATP1A2.** To determine whether β-blockers are OATP1A2 inhibitors, competition studies were performed using rosuvastatin as a probe substrate (Fig. 2). Carvedilol was the only β-blocker able to fully inhibit OATP1A2-mediated uptake of rosuvastatin, and it was the most potent inhibitor, with an  $IC_{50}$  of 3.2 μM. Metoprolol, propranolol, acebutolol, alprenolol,



**Fig. 1.** OATP1A2-mediated transport of rosuvastatin. (A) Uptake of 60 μM rosuvastatin in HEK293-OATP1A2 and HEK293-VC cells was assessed for 3, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 600 seconds at 37°C. (B) Uptake of rosuvastatin (10, 25, 50, 75, 100, and 250 μM) in HEK293-OATP1A2 and HEK293-VC cells was assessed for 2 minutes at 37°C. The quantity of intracellular rosuvastatin was normalized to protein content. To obtain the net transport, values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells.  $K_m$  and  $V_{max}$  were calculated by fitting the data to the Michaelis-Menten equation. Each point represents the mean ± S.D. of triplicate from a single experiment.

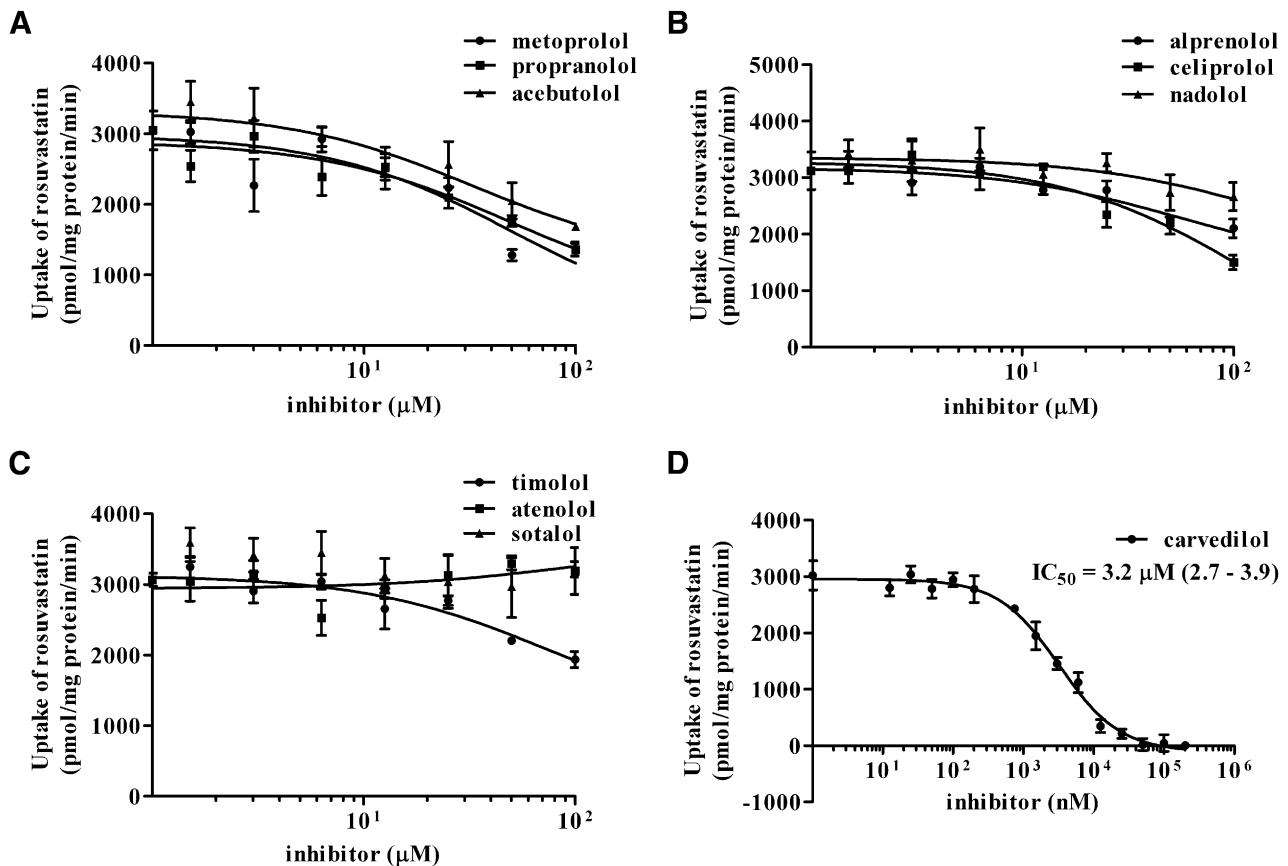
celiprolol, nadolol, and timolol had a small effect on OATP1A2-mediated uptake of rosuvastatin. Given that complete inhibition could not be achieved by these  $\beta$ -blockers at the concentrations tested, the  $IC_{50}$  could not be calculated appropriately. Sotalol and atenolol demonstrated no significant effect on rosuvastatin uptake.

**Effect of Tricyclic Compounds on Rosuvastatin Uptake through OATP1A2.** By observing the structures of all  $\beta$ -blockers tested, it can be noticed that carvedilol differs from the others by the presence of a tricyclic moiety in its structure (Fig. 3; Supplemental Fig. 1A). Inhibition studies were performed with compounds with a similar structure using rosuvastatin as the probe substrate to determine whether the tricyclic ring is responsible for carvedilol's strong inhibitory effect on OATP1A2 (Fig. 3; Supplemental Figs. 1B and 2; Table 1). Carazolol exerted the strongest inhibition, with an  $IC_{50}$  of 3.7  $\mu$ M. The inhibition potencies were followed by clomipramine ( $IC_{50}$  = 8.2  $\mu$ M), amitriptyline ( $IC_{50}$  = 11.7  $\mu$ M), chlorpromazine ( $IC_{50}$  = 12.0  $\mu$ M), doxepin ( $IC_{50}$  = 12.1  $\mu$ M), trimipramine ( $IC_{50}$  = 15.0  $\mu$ M), imipramine ( $IC_{50}$  = 16.9  $\mu$ M), nortriptyline ( $IC_{50}$  = 25.0  $\mu$ M), and desipramine ( $IC_{50}$  = 37.0  $\mu$ M). Carbamazepine, carbazole, and phenothiazine exerted no significant effect on OATP1A2-mediated uptake of rosuvastatin.

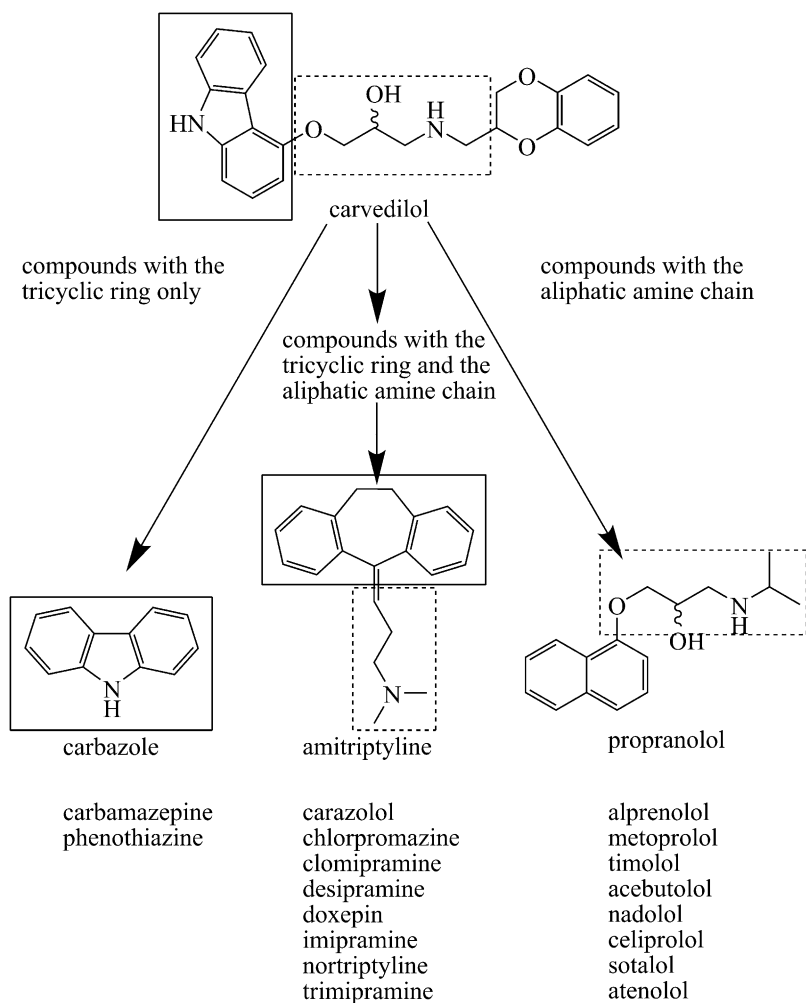
**Transport of Tricyclic Compounds through OATP1A2.** Comparison between the structures of the compounds that inhibited OATP1A2 and those that exerted no effect reveals

that a molecule composed of a tricyclic ring with a short aliphatic amine chain is able to inhibit OATP1A2 activity. As several tricyclic drugs inhibited rosuvastatin uptake, it was relevant to determine if they are also substrates of OATP1A2. Analytical methods were developed for each drug, and transport assays were conducted. Given that carvedilol showed the strongest inhibitory effect on rosuvastatin transport, it was the first tricyclic drug assessed. Incubation of carvedilol with HEK293-OATP1A2 and HEK293-VC showed no difference in intracellular concentrations of carvedilol between the two cell lines (Fig. 4). Thus, carvedilol is not a substrate of OATP1A2 but an inhibitor. Other tricyclic drugs, such as amitriptyline, doxepin, trimipramine, and imipramine, were also evaluated but it was found that none of the drugs were OATP1A2 substrates. Only the results for carvedilol are presented as an example since all graphs were similar.

**Determination of Inhibition Constant of Tricyclic Compounds.** The  $K_i$  of previously identified inhibitors for OATP1A2 transport were determined using rosuvastatin as a probe substrate. A Dixon plot was drawn for each inhibitor, and the  $K_i$  was calculated (Fig. 5; Supplemental Fig. 3; Table 2). Carvedilol showed the lowest  $K_i$  value (1.1  $\mu$ M), implying it as the strongest inhibitor evaluated. The inhibition potencies were followed by trimipramine (2.8  $\mu$ M), carazolol (3.2  $\mu$ M), clomipramine (3.3  $\mu$ M), imipramine (3.5  $\mu$ M), amitriptyline



**Fig. 2.** Inhibition of OATP1A2-mediated transport of rosuvastatin by different  $\beta$ -blockers. HEK293-OATP1A2 and HEK293-VC cells were coincubated with rosuvastatin (150  $\mu$ M) and different  $\beta$ -blockers (1.5–100  $\mu$ M; up to 200  $\mu$ M for carvedilol) for 2 minutes at 37°C. The quantity of intracellular rosuvastatin measured was normalized to protein content. To obtain the net transport, values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells.  $IC_{50}$  values were calculated by fitting the data to the log(inhibitor) versus response equation in GraphPad Prism. (A) ●, metoprolol; ■, propranolol; ▲, acebutolol. (B) ●, alprenolol; ■, celiprolol; ▲, nadolol. (C) ●, timolol; ■, atenolol; ▲, sotalol. (D) ●, carvedilol. Each point represents the mean  $\pm$  S.D. of triplicate from a single experiment. The values in parentheses represent the 95% confidence interval.



**Fig. 3.** Chemical structures of compounds evaluated. Structure of carvedilol, carbazole, amitriptyline, and propranolol and the list of structurally similar compounds evaluated.

(3.7  $\mu\text{M}$ ), doxepin (4.7  $\mu\text{M}$ ), chlorpromazine (5.3  $\mu\text{M}$ ), desipramine (8.4  $\mu\text{M}$ ), and nortriptyline (12.0  $\mu\text{M}$ ).

## Discussion

A method to study drug–drug interactions between substrates of OATP1A2 using rosuvastatin as a probe substrate

TABLE 1

IC<sub>50</sub> values from the inhibition of rosuvastatin uptake through OATP1A2 by different tricyclic compounds

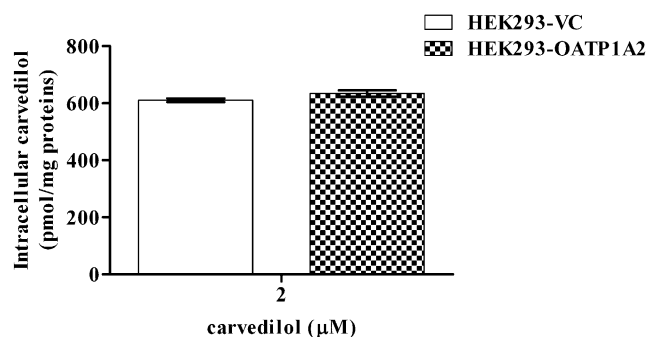
The values in parentheses represent the 95% confidence interval (see also Supplemental Fig. 2).

Compound	IC <sub>50</sub>
	$\mu\text{M}$
Amitriptyline	11.7 (9.1–15.0)
Carazolol	3.7 (2.9–4.8)
Carvedilol	3.2 (2.7–3.9)
Chlorpromazine	12.0 (7.5–19.2)
Clomipramine	8.2 (5.2–13.0)
Desipramine	37.0 (22.3–61.4)
Doxepin	12.1 (7.1–20.6)
Imipramine	16.9 (13.2–21.6)
Nortriptyline	25.0 (13.2–47.6)
Trimipramine	15.0 (8.1–27.8)
Carbamazepine	No effect
Carbazole	No effect
Phenothiazine	No effect

was developed and validated. Inhibition studies between the probe substrate and different  $\beta$ -blockers demonstrated that carvedilol was the most potent inhibitor. The other  $\beta$ -blockers evaluated had little or no significant effects. Furthermore, a structure–activity relationship established from the tricyclic compounds evaluated demonstrated that the transport activity of OATP1A2 was inhibited by compounds composed of a tricyclic ring and short aliphatic amine chain. These compounds were not found to be transported by OATP1A2.

The  $K_m$  of rosuvastatin for OATP1A2 in this study was determined to be 60.2  $\mu\text{M}$ , which is higher than the previously published  $K_m$  (2.6  $\mu\text{M}$ ) (Ho et al., 2006). Our results also show a superior efficiency ( $V_{\text{max}}/K_m$ ) of 161.8 compared with 1  $\mu\text{l}/\text{mg}$  protein per minute (Ho et al., 2006). This discrepancy may be explained by the different in vitro models employed. Ho et al. (2006) used transiently transfected HeLa cells, while a HEK293-OATP1A2 stable cell line was used in this study. The major setback with transiently transfected cells is the lack of reproducibility from one experiment to another. Variability may even arise within a single experiment from one well of transfected cells to another. A stable cell line offers uniformity within a cell population and simplicity once the cell line has been developed. Due to these advantages, a stable cell line was used in this study.

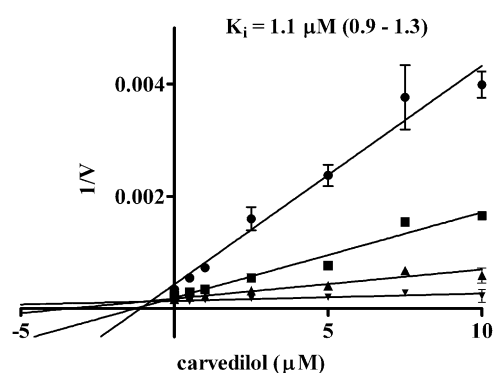
The  $\beta$ -blockers evaluated, with the exception of carvedilol, were not able to compete with rosuvastatin for OATP1A2



**Fig. 4.** Intracellular concentrations of carvedilol. HEK293-OATP1A2 and HEK293-VC cells were incubated in a solution of 2  $\mu\text{M}$  carvedilol for 2 minutes at 37°C. The quantity of intracellular carvedilol was normalized to protein content. Each column represents the mean  $\pm$  S.D. of triplicate from a single experiment.

transport. These results imply that they are either not substrates of OATP1A2, as previously reported, or they have a weaker affinity for the transporter than rosuvastatin. A  $K_m$  of 84.3  $\mu\text{M}$  for nadolol has been reported, which supports the second explanation (Misaka et al., 2014). It remains to be determined for the other  $\beta$ -blockers. Carvedilol blocked OATP1A2-mediated uptake of rosuvastatin very efficiently without being transported by it. Other compounds evaluated with a similar structure also blocked OATP1A2-mediated uptake of rosuvastatin. These results suggest that compounds composed of a tricyclic ring and short aliphatic amine chain could potentially block the transport of OATP1A2 substrates. Based on this structure-relationship finding, tricyclic antidepressants have been selected to further investigate OATP1A2 uptake and transport inhibition. Our results observed with the tricyclic antidepressants strongly suggest that these structural features appear to be determinant for the inhibition of OATP1A2 but not to mediate substrate uptake transport.

Drug–drug interaction studies involving the transporter have mainly focused on drug absorption since OATP1A2



**Fig. 5.** Dixon plot of inhibition of OATP1A2-mediated transport of rosuvastatin by carvedilol. HEK293-OATP1A2 and HEK293-VC cells were coincubated with rosuvastatin (25, 50, 100, and 250  $\mu\text{M}$ ) and carvedilol (0.5–10  $\mu\text{M}$ ) for 2 minutes at 37°C. The quantity of intracellular rosuvastatin was normalized to protein content. To obtain the net transport, the values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells. The x-axis represents the concentration of the inhibitor, and the y-axis represents the reciprocal velocity ( $1/V$ ). Linear regression was used to fit each set of data, and the intercept of all lines represents the  $-K_i$ . The  $K_i$  was accurately calculated in GraphPad Prism. ●, 25  $\mu\text{M}$  rosuvastatin; ■, 50  $\mu\text{M}$  rosuvastatin; ▲, 100  $\mu\text{M}$  rosuvastatin; ▼, 250  $\mu\text{M}$  rosuvastatin. The values in parentheses represent the 95% confidence interval.

expression has previously been detected at the duodenum by immunohistochemistry (Glaeser et al., 2007). Several publications demonstrated that fruit juices and green tea decreased the bioavailability of OATP1A2 substrates (Dresser et al., 2005; Bailey et al., 2007; Glaeser et al., 2007; Rebello et al., 2012; Misaka et al., 2014). However, other publications failed to prove an interaction demonstrated in a cell model in humans (Eechoute et al., 2011). In addition, Misaka et al. (2014) showed that green tea decreased the  $C_{max}$  and AUC of nadolol and their results suggest that the interaction is in part mediated by OATP1A2. In contrast, grapefruit juice, an established inhibitor of OATP1A2, did not have the same effect on nadolol (Misaka et al., 2013). A recent study looking at influx and efflux drug transporters in the small intestine using liquid chromatography–tandem mass spectrometry demonstrated that OATP1A2 was not expressed in any segment of the intestine and other influx transporters, such as OATP2B1, PEPT1, and OCT1, may be implicated in the absorption of drugs instead (Groer et al., 2013; Drozdzik et al., 2014). This may explain the inconsistency in studies where in vivo and in vitro data do not corroborate and different clinical studies using the same inhibitor have conflicting results.

As OATP1A2 is expressed on the luminal membrane of endothelial cells from the blood-brain barrier, it may potentially be involved in the distribution of drugs to the brain. Tricyclic antidepressants must cross the blood-brain barrier to reach their site of action, and we showed that these drugs are inhibitors of OATP1A2. It could be speculated that the coadministration of a tricyclic antidepressant with a central nervous system (CNS) drug substrate for OATP1A2 may lead to a drug–drug interaction when both drugs meet at the blood-brain barrier. It could result in a loss in efficacy by limiting their penetration to the brain. Cheng et al. (2012) conducted a structure-activity relationship study using triptan structural analogs and demonstrated that an amine atom was necessary for efficient uptake through OATP1A2 and that the uptake rate was the highest for a tertiary amine followed by a secondary and then primary amine. Likewise, tricyclic antidepressants, which are also CNS active drugs, share some general similarities with triptans and  $\beta$ -blockers, including an amine residue within their structure, a tricyclic ring, and a short aliphatic chain.

Taken together, the data in this study showed that compounds composed of a tricyclic ring with a short aliphatic amine chain inhibited OATP1A2 activity. Tricyclic antidepressants are a class of medication with such a structure, and

**TABLE 2**

$K_i$  values from the inhibition of rosuvastatin uptake through OATP1A2 by different tricyclic compounds

The values in parentheses represent the 95% confidence interval (see also Supplemental Fig. 3).

Compound	$K_i$ $\mu\text{M}$
Amitriptyline	3.7 (2.9–4.6)
Carazolol	3.2 (2.2–4.1)
Carvedilol	1.1 (0.9–1.3)
Chlorpromazine	5.3 (4.4–6.1)
Clomipramine	3.3 (2.7–3.9)
Desipramine	8.4 (6.7–10.0)
Doxepin	4.7 (3.6–5.8)
Imipramine	3.5 (2.5–4.5)
Nortriptyline	12.0 (9.0–15.0)
Trimipramine	2.8 (2.1–3.5)

we demonstrated their strong inhibition on OATP1A2-mediated transport of rosuvastatin. Such an interaction may potentially be significant for CNS-active drugs that use OATP1A2 to cross the blood-brain barrier. Future work needs to be done to assess whether OATP1A2-mediated transport of CNS-active drugs can be blocked by tricyclic antidepressants. The clinical relevance of such an interaction needs to be investigated further as well.

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#### Authorship Contributions

*Participated in research design:* Lu, Michaud, Turgeon.

*Conducted experiments:* Lu, Guilarte Moya, Leung.

*Contributed new reagents or analytic tools:* Gaudette.

*Performed data analysis:* Lu, Gaudette.

*Wrote or contributed to the writing of the manuscript:* Lu, Michaud, Turgeon.

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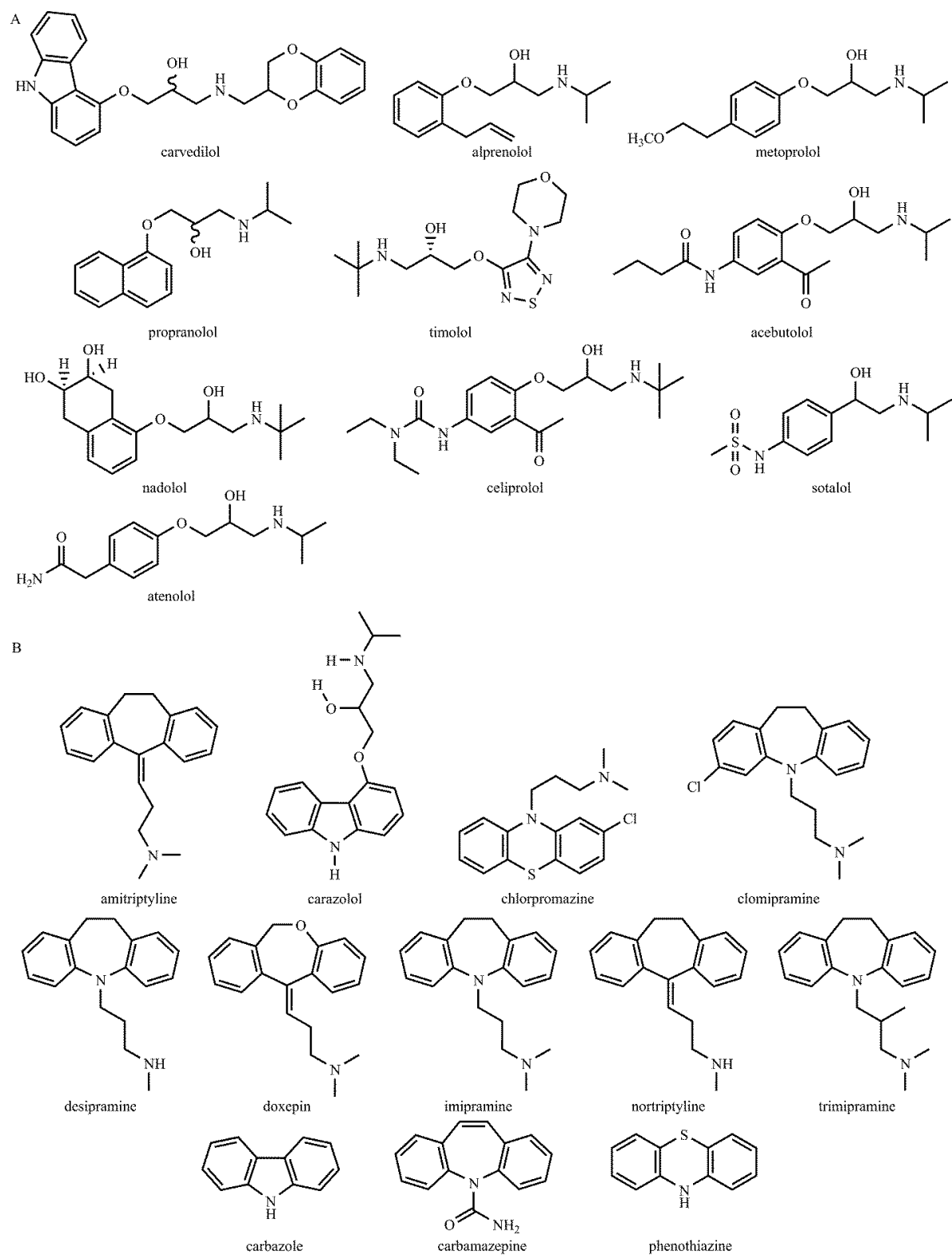
## **Supplemental Figures**

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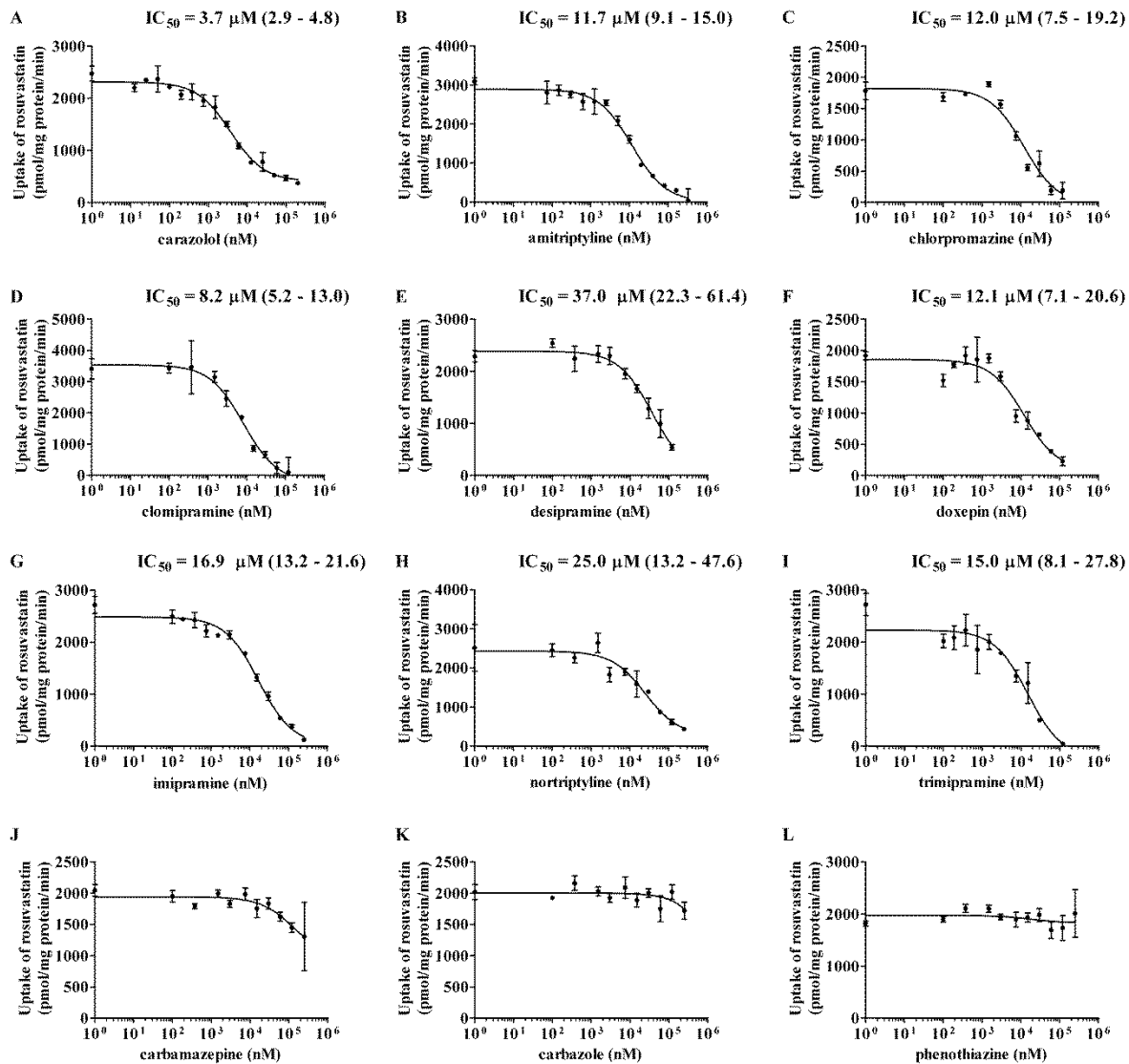
#### **Effects of $\beta$ -blockers and tricyclic antidepressants on the activity of human organic anion transporting polypeptide 1A2 (OATP1A2)**

Jennifer Lu, Veronique Michaud, Liliam Gabriela Guilarte Moya, Fleur Gaudette, Yat Hei Leung, and Jacques Turgeon



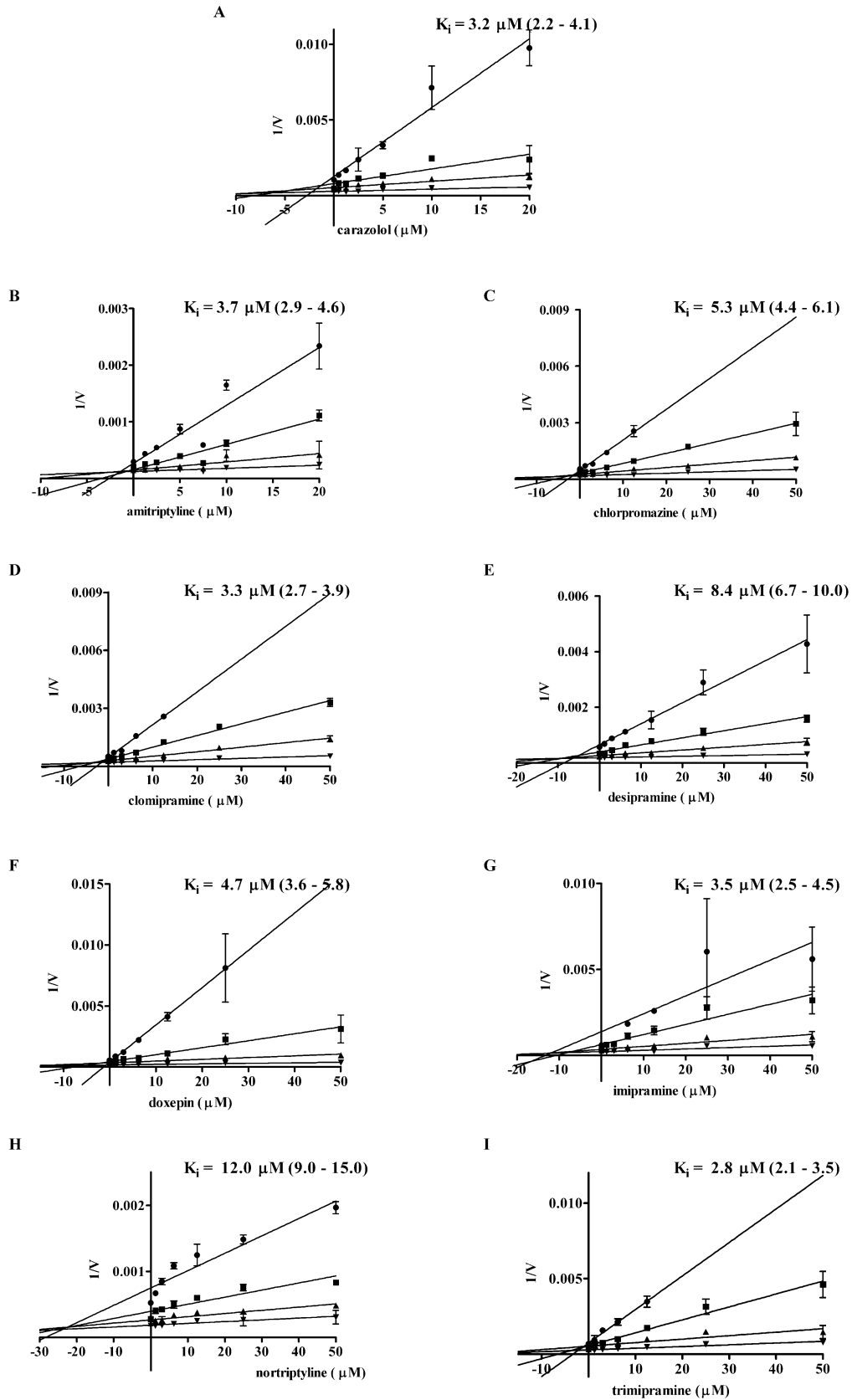


**Supplemental Figure 1. Structures of (A) different  $\beta$ -blockers tested and (B) different tricyclic compounds tested.**



**Supplemental Figure 2. Inhibition of OATP1A2-mediated transport of rosuvastatin by different tricyclic compounds.** HEK293-OATP1A2 and HEK293-VC cells were coincubated with rosuvastatin (150  $\mu M$ ) and different tricyclic compounds (12.5 nM – 250  $\mu M$ ) for 2 min at 37°C. The quantity of intracellular rosuvastatin was normalized to protein content. To obtain the net transport, values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells.  $IC_{50}$  values were calculated by fitting the data to the log(inhibitor)

vs. response equation in GraphPad Prism. (A) carazolol; (B) amitriptyline; (C) chlorpromazine; (D) clomipramine; (E) desipramine; (F) doxepin; (G) imipramine; (H) nortriptyline; (I) trimipramine; (J) carbamazepine; (K) carbazole; and (L) phenothiazine. Each point represents the mean  $\pm$  S.D. of triplicate from a single experiment. The values in parentheses represent the 95% confidence interval.



**Supplemental Figure 3. Dixon plots of inhibition of OATP1A2-mediated transport of rosuvastatin by different tricyclic compounds.** HEK293-OATP1A2 and HEK293-VC cells were coincubated with rosuvastatin (25, 50, 100, 250  $\mu\text{M}$ ) and different tricyclic drugs (0.5 – 50  $\mu\text{M}$ ) for 2 min at 37°C. The quantity of intracellular rosuvastatin was normalized to protein content. To obtain the net transport, values measured in the VC cells were subtracted from the values measured in OATP1A2-expressed cells. The x-axis represents the concentration of the inhibitor and the y-axis represents the reciprocal velocity ( $1/V$ ). Linear regression was used to fit each set of data and the intercept of all lines represents the  $-K_i$ . The  $K_i$  was accurately calculated in GraphPad Prism. (●) 25  $\mu\text{M}$  rosuvastatin; (■) 50  $\mu\text{M}$  rosuvastatin; (▲) 100  $\mu\text{M}$  rosuvastatin; (▼) 250  $\mu\text{M}$  rosuvastatin. (A) carazolol; (B) amitriptyline; (C) chlorpromazine; (D) clomipramine; (E) desipramine; (F) doxepin; (G) imipramine; (H) nortriptyline; and (I) trimipramine. The values in parentheses represent the 95% confidence interval.