DMD Fast Forward. Published on September 9, 2009 as doi:10.1124/dmd.109.028522

DMD 28522

Interaction of Macrolide Antibiotics with Intestinally Expressed Human and Rat Organic Anion Transporting Polypeptides

Tian Lan, Anuradha Rao, Jamie Haywood, Charles B. Davis, Chao Han, Eric Garver, Paul A. Dawson

Department of Internal Medicine, Section on Gastroenterology, Wake Forest University School of Medicine. Medical Center Blvd., Winston-Salem, NC 27157, (T.L., A.R., J.H., P.A.D) and Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Collegeville, Pennsylvania (C.B.D., C.H., E.G.)

Running Title: OATP interactions with Macrolide Antibiotics

Corresponding Author:

Paul A. Dawson, Ph.D.

Department of Internal Medicine, Section on Gastroenterology

Wake Forest University School of Medicine

Medical Center Blvd., Winston-Salem, NC 27157 USA

Telephone: (336) 716-4633

Fax: (336) 716-6376

E-mail: pdawson@wfubmc.edu

Number of text pages: 26

Number of tables: 0

Number of figures: 6 (plus 4 figures as supplemental data)

Number of references: 40

Number of words used in the Abstract: 208

Number of words in the Introduction: 661

Number of words in the Discussion: 614

Non-standard Abbreviations: ADME, Absorption, Distribution, Metabolism, and Excretion; AUC, area under the blood-concentration time curve; Azi, azithromycin; BSP, bromosulfophthalein; CI, confidence interval; Clari, clarithromycin; E3S, estrone-3-sulfate; Ery, erythromycin; MDCK, Madin Darby Canine Kidney; OATP (human) or Oatp (rodent), organic anion transporting polypeptide; Rif, rifamycin SV.

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

The macrolide antibiotics azithromycin and clarithromycin are large molecular weight compounds that exhibit moderate to excellent oral bioavailability in preclinical species and humans. Previous concomitant dosing studies in rats using rifamycin SV, a general organic anion transporting polypeptide (OATP) inhibitor, suggested that the high oral absorption of azithromycin and clarithromycin may be due to facilitative uptake by intestinal Oatps. In this study, we used OATP/Oatp-expressing cells to investigate the interaction of macrolides with rat Oatp1a5, human OATP1A2, and human/rat OATP2B1/Oatp2b1. These experiments demonstrated that azithromycin and clarithromycin were potent inhibitors of rat Oatp1a5-mediated taurocholate uptake with apparent inhibitor constant (K_i) values of 3.3 μM and 2.4 μM, respectively. The macrolides functioned as noncompetitive inhibitors, but were not transport substrates for rat Oatp1a5, as assessed by direct uptake measurements of radiolabeled azithromycin and clarithromycin. *Cis*-inhibition and direct uptake studies further demonstrated that azithromycin and clarithromycin were only very weak inhibitors and not substrates for human OATP1A2 and human/rat OATP2B1/Oatp2b1. In summary, these results indicate that the macrolides azithromycin and clarithromycin potently inhibit rat oatp1a5, but do not significantly interact with OATP1A2 and OATP2B1/Oatp2b1. These intestinally expressed OATP/Oatp(s) are not responsible for the postulated facilitative uptake of azithromycin and clarithromycin and alternative facilitative pathways must exist for their intestinal absorption.

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Macrolide antibiotics such as erythromycin, clarithromycin and azithromycin can alter drug metabolism by several mechanisms that includes inhibiting CYP3A4 (Ito et al., 2003; Polasek and Miners, 2006) and P-glycoprotein (Kim et al., 1999; Marzolini et al., 2004) in the intestine and liver. Another potential mechanism is through direct interactions with members of the Organic Anion Transporting Polypeptide (OATP/*SLCO*) family of transporters. The OATPs comprise a superfamily of sodium-independent transporters that facilitate transport of endogenous compounds and structurally diverse xenobiotics (Kim, 2003; Hagenbuch and Meier, 2004). The OATP/*SLCO* family includes 11 human and 15 rodent genes, which are widely expressed in many tissues, including those responsible for mediating the ADME properties of drugs such as the liver, kidney, blood-brain barrier, and intestine (Hagenbuch and Gui, 2008). A variety of drugs are transported by members of the OATP/*SLCO* family, including statins (cerivastatin, fluvastatin, pitavastatin, pravastatin, rousuvastatin), benzylpencillin, digoxin, fexofenadine, methotrexate, and rifampicin (Hagenbuch and Meier, 2003; Konig et al., 2006). In addition, certain drugs and nutritional supplements, while not OATP substrates themselves, can act as inhibitors of OATP-mediated transport, thereby increasing the potential for significant drug-drug or drug-nutrient interactions (Fuchikami et al., 2006; Konig et al., 2006; Poirier et al., 2007). These safety concerns are underscored by the recent identification of common variants of *SLCO1B1* that are strongly associated with an increased risk of statin-induced myopathy (Link et al., 2008).

Previous studies have demonstrated a significant role for the hepatic OATPs, OATP1B1 and OATP1B3, in drug metabolism (Smith et al., 2005; Ho et al., 2006; Ishiguro et al., 2006; Seithel et al., 2007). Competition for OATP-mediated intestinal absorption may influence drug disposition and represents a potential mechanism for significant drug interactions. Among the OATP/*SLCO* family members expressed in small intestine, efforts have concentrated on human OATP1A2 (OATP-A; gene symbol *SLCO1A2*) and OATP2B1 (OATP-B; gene symbol

SLCO2B1), as well as rat Oatp1a5 (Oatp3; gene symbol *Slco1a5*) (Kobayashi et al., 2003; Kikuchi et al., 2006; Glaeser et al., 2007; Tani et al., 2008). While first identified in liver, *SLCO1A2* mRNA is expressed at higher levels in brain, kidney, testis and intestine. Furthermore, OATP1A2 protein is expressed at the apical brush border membrane of human small intestinal epithelial cells, and compelling evidence supports a role for OATP1A2 in the intestinal absorption of fexofenadine (Dresser et al., 2002; Glaeser et al., 2007) and the quinoline antibiotics (Maeda et al., 2007). *SLCO2B1* mRNA is abundantly expressed in various regions of human small intestine (Nishimura and Naito, 2005; Englund et al., 2006; Seithel et al., 2006; Meier et al., 2007). Immunohistochemical studies localized OATP2B1 protein to the enterocyte apical brush border membrane in human small intestine, where it may be important for pravastatin absorption (Kobayashi et al., 2003). In the rat, *Slco1a5* mRNA is expressed at low levels down the length of the small intestine and Oatp1a5 protein was detected on the apical brush border membrane of jejunal enterocytes (Walters et al., 2000). Rat Oatp1a5 directly transports fexofenadine and the β-adrenergic receptor antagonist Talinolol with similar affinities to that observed for rat isolated intestinal tissue (Kikuchi et al., 2006; Shirasaka et al., 2009).

Azithromycin and clarithromycin are commonly used macrolide antibiotics with good oral bioavailability in humans of approximately 37% and 52%, respectively (Foulds et al., 1990; Chu et al., 1992), despite their large molecular weights, high hydrogen-bonding potential, and interactions with P-glycoprotein (azithromycin and clarithromycin) and CYP3A4 (clarithromycin) (Kim et al., 1999; Lipinski et al., 2001; Polasek and Miners, 2006). Previously, we showed that concomitant dosing of rifamycin SV, a general OATP/Oatp inhibitor (Vavricka et al., 2002), significantly reduced the oral AUC for azithromycin and clarithromycin in rats (Garver et al., 2008). Additional *in vivo* studies suggested that the reduced AUC was not due to increased blood clearance and *in vitro* studies demonstrated an interaction of the macrolides with rat Oatp1a5. The present study was designed to determine whether Oatp1a5 and/or other well

characterized intestinally expressed human/rodent OATP/Oatps are potentially involved in the absorption of these macrolide antibiotics.

Materials and Methods

Materials. [^{3}H]taurocholic acid (5.0 Ci/mmol) and [^{3}H]estrone-3-sulfate (57.3 Ci/mmol) were purchased from PerkinElmer, Inc. (Boston, MA), [^{3}H]azithromycin (80 Ci/mmol), [^{3}H]clarithromyin (80 Ci/mmol) were purchased from American Radiolabeled Chemicals, Inc (St. Louis, MO). Madin-Darby Canine Kidney Cells (MDCK) cells were obtained from the American Type Culture Collection and grown in monolayer at 37°C in an atmosphere of 5% CO_2. The MDCK-rat Oatp1a5 inducible cell line was generated as described (Walters et al., 2000). Oatp1a5-transport activity is undetectable under basal conditions, but is dramatically induced by prior incubation with sodium butyrate (Walters et al., 2000). Azithromycin (Azi) was purchased from Pliva Croatia Ltd. (Zagreb, Croatia), and clarithromycin (Clari) was purchased from Apin Chemicals, Ltd. (Abingdon, Oxon, UK). Erythromycin (Ery), rifamycin SV sodium salt (Rif), bromosulfothalein (BSP), sodium butyrate, and taurocholate were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Phosphate-buffered saline (PBS), Dulbecco's modified Eagle's Medium, 10% (v/v) fetal calf serum, penicillin, streptomycin, and G-418 sulfate, were purchased from Invitrogen (Carlsbad, CA).

Rodent and Human Transporter Expression Plasmids. The rat Oatp2b1 expression plasmid was constructed as follows. A pair of oligonucleotide primers, 5'GGAAGATCTCACCTGAGGCA-AGTGTGTGAC3' and 5'CAAAGGCTCAGAAGATGCATGGCTGTG3', corresponding to rat *Slco2b1* nucleotides 24 to 53 and 2223 to 2197, respectively, was used to PCR-amplify the rat *Slco2b1* from Sprague-Dawley rat liver cDNA using previously described conditions (Walters et al., 2000). The 2200 base pair product was subcloned into a pGEM-T-Easy vector (Promega) and then transferred to the pcDNA3 expression vector (Invitrogen) for transfection experiments.

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

The human OATP1A2 (variant 1; accession number NM_134431.1, catalog number SC309088) and OATP2B1 (catalog number SC115618) expression plasmids were obtained from Origene Technologies. The mouse Oatp2b1 plasmid (I.M.A.G.E. cDNA clone 5101188) was obtained from Open Biosystems; the mouse Oatp2b1 expression plasmid includes 101 nucleotides of 5' untranslated region, the full-length 2052 nucleotide coding region, and 183 nucleotide of 3' untranslated region. The cDNA inserts for all transporter expression plasmids were verified by DNA sequencing.

Transport Assays in Stably Transfected MDCK cells and Transiently Transfected COS Cells. MDCK and MDCK-rat Oatp1a5 cells were maintained in DMEM containing 1000 mg/L D-glucose, 10% (v/v) fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Medium B) or Medium B containing 350 µg/ml G418, respectively. For determination of the EC_{50} and K_i values, MDCK or MDCK-rat Oatp1a5 cells were seeded onto 24-well plates at 1.3 X 10^5 and 6.5 X 10^4 cells per well, respectively. On day 2, the cells were refed the same media (MDCK) or the media containing 10 mM sodium butyrate to induce expression of Oatp1a5 (MDCK-rat Oatp1a5). On day 3, the cells were washed with pre-warmed PBS and incubated at 37°C in triplicate with DMEM (without antibiotics and calf serum) containing the indicated concentration of [^{3}H]taurocholate plus vehicle or competitor. After 10 min at 37°C, the cell monolayers were washed three times with ice cold PBS and processed to determine cell-associated protein and radioactivity as described previously (Walters et al., 2000; Garver et al., 2008). Uptake values were corrected for background at each concentration of solute or competitor by subtracting the uptake values from parallel plates of parental MDCK cells. The K_i was estimated by measuring the uptake at several fixed concentrations of [^{3}H]taurocholate and competitors (Dixon, 1953).

COS-1 cells were maintained in DMEM plus 4500 mg/L glucose, 10% (v/v) fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Medium A). On day 0, cells were seeded onto 100 mm dishes at 2 X 10^6 cells per 100 mm dish. On day 1, the cells were

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

transfected with 24 μg of Oatp/OATP expression plasmid or 24 μg of a mixture of control plasmids that included 10 μg of pEYFP-C1 (Clontech) and 14 μg pcDNA3.1/Hygro(+) plasmid (Invitrogen) using Lipofectamine Transfection Reagent (Invitrogen). On day 2, transfected cells were trypsinized, re-seeded onto 24-well plates at 2 X 10^5 cells per well. On day 4, cells were washed and pre-incubated for 30 min at 37° C in uptake buffer that included 125 mM NaCl, 48 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 12 mM $MgSO_4$ and 25 mM (MES) 2-(4-Morpholino)ethanesulfonic acid, (pH 6.0 buffer) or 25 mM (HEPES) (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (pH 7.4 buffer) (Kobayashi et al., 2003). The cells were then washed and incubated at 37°C with the indicated radiolabeled solute in the presence of vehicle or unlabeled competitor. After 10 min at 37°C, the cell monolayers were washed three times with ice cold PBS and processed to determine cell-associated protein and radioactivity (Walters et al., 2000; Garver et al., 2008). Uptake was linear over this period and the values were corrected for the background at each concentration of solute or competitor by subtracting the uptake values from parallel plates of control plasmid-transfected COS cells.

Data Analysis. All data with the exception of the EC_{50} values are expressed as mean ± SEM. The EC_{50} values, the concentration of competitor required to inhibit 50% of specific solute uptake, were calculated by fitting the data to a sigmoidal dose-response curve using GraphPad Prism (GraphPad Software) and are expressed as the mean and 95% confidence intervals. Statistical analysis was performed using the Student's paired *t*-test with $P < 0.05$ as the criterion for significance.

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Inhibition of Taurocholate Uptake by Macrolides in Rat Oatp1a5-expressing MDCK Cells. Previous studies showed that azithromycin and clarithromycin inhibited [^{3}H]taurocholate uptake by MDCK-rat Oatp1a5 expressing cells (Garver et al., 2008). In order to further quantify macrolide interactions with Oatp1a5, additional dose-response inhibition studies were performed. As shown in Fig. 1, after addition of increasing concentrations up to 100 μM, azithromycin, clarithromycin and erythromycin all showed a clear dose-dependent inhibition of rat Oatp1a5-mediated taurocholate uptake. The macrolides were similarly effective, with calculated mean EC_{50} values of 2.0 μM (1.4 - 2.7), 2.9 μM (2.3 - 3.6), and 5.3 μM (3.6 - 7.8) (95% CI) for azithromycin, clarithromycin, and erythromycin, respectively.

In order to gain insight into the mechanism underlying the inhibition of rat Oatp1a5 by macrolides, the transport activity of MDCK-rat Oatp1a5 cells was analyzed at several concentrations of [^{3}H]taurocholate and unlabeled azithromycin or clarithromycin. The results were then subjected to Dixon plot analysis to derive an apparent inhibition constant (K_i) and to determine the apparent mode of inhibition. Analysis of the fitted data (Fig. 2) revealed potent inhibition of rat Oatp1a5-mediated taurocholate uptake, with apparent K_i values of approximately 3.3 $\pm$ 0.4 and 2.4 $\pm$ 0.3 μM for azithromycin and clarithromycin, respectively. The analysis was consistent with a noncompetitive mode of inhibition for each macrolide.

Macrolide Interaction with Human OATP1A2 Expressed in Transfected COS Cells. In order to begin identifying human intestinally expressed OATPs that interact with the macrolides, *cis*-inhibition studies were performed using human OATP1A2-transfected COS cells. As one of the goals of these studies was to examine the interaction of macrolides with OATPs under conditions that mimic the surface microclimate of the small intestine (Lucas, 1983), pilot studies were performed to examine the effect of pH on OATP1A2-mediated transport in transfected COS cells. Similar to previous studies that demonstrated a pH-dependence for methotrexate

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

transport by human OATP1A2-expressing *X. laevis* oocytes (Badagnani et al., 2006), OATP1A2-mediated [^{3}H]taurocholate or [^{3}H]estrone-3-sulfate uptake in transfected COS cells was increased at pH 6.0 versus pH 7.4 (Supplemental Data, Fig. 7). Similar increases in radiolabeled taurocholate or estrone-3-sulfate uptake were observed for rat Oatp1a5 and human OATP2B1 (Supplemental Data, Fig. 7). Based on previous reports demonstrating increased transport at acidic pH by the intestinally expressed OATPs/Oatps, including OATP1A2, OATP2B1, and Oatp1a5 (Kobayashi et al., 2003; Nozawa et al., 2004; Badagnani et al., 2006; Tani et al., 2008) and our pilot data (Fig. 7), subsequent uptake studies were carried out under the pH 6.0 conditions.

As shown in Fig 3A, uptake of 5 µM [^{3}H]estrone-3-sulfate in the presence of 250 µM of the indicated competitor was analyzed using human OATP1A2-transfected COS cells. Whereas BSP and rifamycin SV strongly inhibited estrone-3-sulfate uptake by approximately 90 and 80% respectively, the macrolides showed only weak inhibition. In order to further quantify the macrolide interactions with OATP1A2, additional dose-response inhibition studies were performed using azithromycin, clarithromycin, erythromycin, and BSP. After addition of increasing concentrations up to 500 µM, azithromycin, clarithromycin, and erythromycin showed little inhibition of 1 µM [^{3}H]estrone-3-sulfate uptake by human OATP1A2 (Figs. 3B to 3D). In contrast, BSP showed a clear dose-dependent inhibition of human OATP1A2-mediated estrone-3-sulfate uptake by the transfected COS cells (Fig. 3E) with a calculated EC_{50} value of approximately 3.8 µM (2.6 - 5.0; 95% CI).

Direct Uptake Studies of Azithromycin and Clarithromycin using Rat Oatp1a5 and Human OATP1A2-transfected Cells. The *cis*-inhibition studies demonstrated that the macrolides are potent inhibitors of rat Oatp1a5 but not human OATP1A2-mediated transport. To clarify whether the macrolides are also substrates for rat Oatp1a5 and further explore their interaction with OATP1A2, direct uptake experiments were performed using [^{3}H]azithromycin and

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

[^{3}H]clarithromycin. Pilot studies were initially performed using the MDCK-rat Oatp1a5 cells. As shown in Fig. 8 (Supplemental Data), rat Oatp1a5 exhibited significant [^{3}H]taurocholate uptake that was approximately 20-fold over background and only an extremely low level of [^{3}H]azithromycin uptake that was approximately 30% over background. Attempts to examine the uptake of [^{3}H]clarithromycin using the MDCK-rat Oatp1a5 cells were unsuccessful due to a very high background level of clarithromycin binding/uptake for the Oatp1a5-expressing and Oatp1a5-negative MDCK cells (Supplemental Data, Fig. 8C). However, evaluation of other cell expression systems indicated that transfected COS cells exhibited an acceptable background for radiolabeled macrolide uptake. In transfected COS cells, rat Oatp1a5 exhibited significant uptake of 5 µM [^{3}H]estrone-3-sulfate, a prototypical low affinity Oatp1a5 substrate (Yarim et al., 2005), that was approximately 9-fold over background. However, there was no appreciable uptake of 10 µM [^{3}H]azithromycin or [^{3}H]clarithromycin over background (Fig. 4A). In addition, studies using increasing concentrations of [^{3}H]clarithromycin up to 200 µM showed only background levels of uptake by the rat Oatp1a5-transfected COS cells (data not shown). In studies using OATP1A2-transfected COS cells, there was also significant uptake of [^{3}H]estrone-3-sulfate that was approximately 2-fold over background, but no uptake of 10 µM [^{3}H]azithromycin or [^{3}H]clarithromycin over background (Fig. 4B).

Interaction of Azithromycin and Clarithromycin with Human OATP2B1 and Rat Oatp2b1 Expressed in transfected COS Cells. In order to identify other intestinally expressed OATPs/Oatps that interact with the macrolides, additional *cis*-inhibition studies were performed using human and rodent OATP2B1/Oatp2b1-transfected cells. The uptake of 5 µM [^{3}H]estrone-3-sulfate in the presence of 250 µM of the indicated competitor was analyzed using human/rat OATP2B1/Oatp2b1-transfected COS cells. Whereas BSP and rifamycin SV strongly inhibited OATP2B1-mediated estrone-3-sulfate uptake by approximately 90 and 85%, respectively, the macrolides showed only weak inhibition (Fig. 5A). Rat Oatp2b1-mediated estrone-3-sulfate

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

uptake was strongly inhibited by BSP, weakly inhibited by rifamycin SV, and unaffected by azithromycin or clarithromycin (Fig. 5B). A similar lack of interaction with the macrolides was observed for mouse Oatp2b1-transfected COS cells (Supplemental Data, Fig. 9). The weak potential interaction of the macrolides with human OATP2B1 was further examined in dose-response inhibition studies. After addition of increasing concentrations up to 500 μM, azithromycin, clarithromycin, and erythromycin showed little inhibition of 1 μM [^{3}H]estrone-3-sulfate uptake by human OATP2B1 (Fig. 6A to 6C). In contrast, BSP showed a clear dose-dependent inhibition of human OATP2B1-mediated estrone-3-sulfate uptake by the transfected COS cells (Fig. 6D) with a calculated EC_{50} value of approximately 1.7 μM (1.1 - 2.3; 95% CI).

While the *cis*-inhibition studies suggested that the macrolides are poor inhibitors of human/rat OATP2B1/Oatp2b1-mediated transport, additional direct uptake studies were also performed to further explore these potential interactions. In transfected COS cells, human OATP2B1 and rat Oatp2b1 exhibited significant uptake of 5 μM [^{3}H]estrone-3-sulfate that was approximately 8-fold and 1.7-fold over background, respectively. In contrast to the estrone-3-sulfate, there was no appreciable uptake of [^{3}H]azithromycin or [^{3}H]clarithromycin over background (Supplemental data; Fig. 10).

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

The macrolide antibiotics azithromycin and clarithromycin exhibit undesirable physicochemical (*e.g.,* large molecular weight and extensive potential for hydrogen bonding) and biopharmaceutical (*e.g.,* substrates for efflux transporter P-gp) properties, but show moderate to excellent oral bioavailability in preclinical species and humans. Among the potential explanations for this paradox is that intestinal transporters facilitate macrolide absorption. Previous studies in rats suggested that oral absorption of azithromycin and clarithromycin is mediated by an Oatp and/or other rifamycin SV-sensitive intestinal transporter (Garver et al., 2008). The present study was designed to identify candidate OATP/Oatps that mediate the oral absorption of macrolide antibiotics. The major findings of this study are that the macrolide antibiotics are potent inhibitors but not substrates of rat Oatp1a5, and that the macrolides interact only weakly with human OATP1A2 and human/rat OATP2B1/Oatp2b1, other well-characterized members of the OATP/Oatp family that are expressed in small intestine.

While interaction of macrolide and ketolide antibiotics with human OATP1B1 and OATP1B3 has been studied (Seithel et al., 2007), the interaction of macrolides with intestinally-expressed OATP/Oatps has not been fully investigated. Direct uptake and *cis*-inhibition experiments were performed using rat Oatp1a5 expressed in stably transfected MDCK cells and transiently transfected COS cells. As previously suggested (Garver et al., 2008), we found a strong dose-dependent inhibition of Oatp1a5-mediated transport by the macrolides. To further understand the mechanism of Oatp1a5 inhibition, transport was examined using various concentrations of [^{3}H]taurocholate and unlabeled macrolide. The study revealed that azithromycin and clarithromycin potently inhibited Oatp1a5-mediated [^{3}H]taurocholate uptake in a noncompetitive fashion with apparent K_i values of 3.3 $\pm$ 0.4 μM and 2.4 $\pm$ 0.3 μM, respectively. The finding that the macrolides were not competitive-type inhibitors was further supported by direct uptake experiments using radiolabeled substrates. While rat Oatp1a5 exhibited robust

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

[^{3}H]taurocholate and [^{3}H]estrone-3-sulfate uptake, there was no significant [^{3}H]azithromycin and [^{3}H]clarithromycin uptake over background.

Another major intestinally expressed OATP/SLCO family member is human OATP1A2. In contrast to their potent interaction with rat Oatp1a5, the macrolides did not inhibit human OATP1A2-mediated transport. The identification of a compound selective for rat Oatp1a5 versus OATP1A2 is not without precedence. OATP1A2 and Oatp1a5 share 72% amino acid identity and transport many common substrates such as taurocholate, estrone-3-sulfate, fexofenadine, and BSP (Hagenbuch and Meier, 2003). However there are examples of compounds such as methotrexate, which are not shared substrates and selectively interact with one but not the other carrier (Cattori et al., 2001; Ohtsuki et al., 2003; Badagnani et al., 2006). In addition to OATP1A2, OATP2B1 is expressed on the apical brush border membrane of the human intestine and is believed to have a role in drug absorption (Kobayashi et al., 2003; Nozawa et al., 2004). As was found for OATP1A2, azithromycin and clarithromycin did not significantly inhibit OATP2B1-mediated uptake of estrone-3-sulfate, a prototypical substrate. The rat and mouse Oatp2b1, which share ~77% amino acid identity with human OATP2B1, showed a similar lack of interaction with azithromycin and clarithromycin. In addition, no significant transport of azithromycin or clarithromycin was observed in direct uptake studies using COS cells transfected with OATP1A2 or human/rat OATP2B1/Oatp2b1.

In conclusion, these studies show that azithromycin and clarithromycin are not efficiently transported by the major characterized intestinally expressed OATP/Oatps, including Oatp1a5, OATP1A2, and OATP2B1/Oatp2b1. Since the inhibition of OATP/Oatp-mediated transport by rifamycin SV is well documented, it is possible that the decreased oral exposure of the macrolides following co-administration of rifamycin SV is due to inhibition of other members of the OATP/Oatp family such as OATP3A1 and OATP4A1 that may be expressed in the small intestine. In addition, since it is unlikely that rifamycin SV inhibits only OATP/Oatp transporters,

it is possible that other non-Oatp uptake transporters are involved in the intestinal absorption of the macrolides.

References

Badagnani I, Castro RA, Taylor TR, Brett CM, Huang CC, Stryke D, Kawamoto M, Johns SJ, Ferrin TE, Carlson EJ, Burchard EG and Giacomini KM (2006) Interaction of methotrexate with organic-anion transporting polypeptide 1A2 and its genetic variants. *J Pharmacol Exp Ther* **318:**521-529.

Cattori V, van Montfoort JE, Stieger B, Landmann L, Meijer DK, Winterhalter KH, Meier PJ and Hagenbuch B (2001) Localization of organic anion transporting polypeptide 4 (Oatp4) in rat liver and comparison of its substrate specificity with Oatp1, Oatp2 and Oatp3. *Pflugers Arch* **443:**188-195.

Chu SY, Deaton R and Cavanaugh J (1992) Absolute bioavailability of clarithromycin after oral administration in humans. *Antimicrob Agents Chemother* **36:**1147-1150.

Dixon M (1953) The determination of enzyme inhibitor constants. *Biochem J* **55:**170-171.

Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ and Kim RB (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* **71:**11-20.

Englund G, Rorsman F, Ronnblom A, Karlbom U, Lazorova L, Grasjo J, Kindmark A and Artursson P (2006) Regional levels of drug transporters along the human intestinal tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells. *Eur J Pharm Sci* **29:**269-277.

Foulds G, Shepard RM and Johnson RB (1990) The pharmacokinetics of azithromycin in human serum and tissues. *J Antimicrob Chemother* **25 Suppl A:**73-82.

Fuchikami H, Satoh H, Tsujimoto M, Ohdo S, Ohtani H and Sawada Y (2006) Effects of herbal extracts on the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos* **34:**577-582.

Garver E, Hugger ED, Shearn SP, Rao A, Dawson PA, Davis CB and Han C (2008) Involvement of intestinal uptake transporters in the absorption of azithromycin and clarithromycin in the rat. *Drug Metab Dispos* **36:**2492-2498.

Glaeser H, Bailey DG, Dresser GK, Gregor JC, Schwarz UI, McGrath JS, Jolicoeur E, Lee W, Leake BF, Tirona RG and Kim RB (2007) Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin Pharmacol Ther* **81:**362-370.

Hagenbuch B and Gui C (2008) Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica* **38:**778-801.

Hagenbuch B and Meier PJ (2003) The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* **1609:**1-18.

Hagenbuch B and Meier PJ (2004) Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch* **447:**653-665.

Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y and Kim RB (2006) Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* **130:**1793-1806.

Ishiguro N, Maeda K, Kishimoto W, Saito A, Harada A, Ebner T, Roth W, Igarashi T and Sugiyama Y (2006) Predominant contribution of OATP1B3 to the hepatic uptake of telmisartan, an angiotensin II receptor antagonist, in humans. *Drug Metab Dispos* **34:**1109-1115.

Ito K, Ogihara K, Kanamitsu S and Itoh T (2003) Prediction of the in vivo interaction between midazolam and macrolides based on in vitro studies using human liver microsomes. *Drug Metab Dispos* **31:**945-954.

Kikuchi A, Nozawa T, Wakasawa T, Maeda T and Tamai I (2006) Transporter-mediated intestinal absorption of fexofenadine in rats. *Drug Metab Pharmacokinet* **21:**308-314.

Kim RB (2003) Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur J Clin Invest* **33 Suppl 2:**1-5.

Kim RB, Wandel C, Leake B, Cvetkovic M, Fromm MF, Dempsey PJ, Roden MM, Belas F, Chaudhary AK, Roden DM, Wood AJ and Wilkinson GR (1999) Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm Res* **16:**408-414.

Kobayashi D, Nozawa T, Imai K, Nezu J, Tsuji A and Tamai I (2003) Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J Pharmacol Exp Ther* **306:**703-708.

Konig J, Seithel A, Gradhand U and Fromm MF (2006) Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol* **372:**432-443.

Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M and Collins R (2008) SLCO1B1 variants and statin-induced myopathy--a genomewide study. *N Engl J Med* **359:**789-799.

Lipinski CA, Lombardo F, Dominy BW and Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46:**3-26.

Lucas M (1983) Determination of acid surface pH in vivo in rat proximal jejunum. *Gut* **24:**734-739.

Maeda T, Takahashi K, Ohtsu N, Oguma T, Ohnishi T, Atsumi R and Tamai I (2007) Identification of influx transporter for the quinolone antibacterial agent levofloxacin. *Mol Pharm* **4:**85-94.

Marzolini C, Paus E, Buclin T and Kim RB (2004) Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* **75:**13-33.

Meier Y, Eloranta JJ, Darimont J, Ismair MG, Hiller C, Fried M, Kullak-Ublick GA and Vavricka SR (2007) Regional distribution of solute carrier mRNA expression along the human intestinal tract. *Drug Metab Dispos* **35:**590-594.

Nishimura M and Naito S (2005) Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab Pharmacokinet* **20:**452-477.

Nozawa T, Imai K, Nezu J, Tsuji A and Tamai I (2004) Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J Pharmacol Exp Ther* **308:**438-445.

Ohtsuki S, Takizawa T, Takanaga H, Terasaki N, Kitazawa T, Sasaki M, Abe T, Hosoya K and Terasaki T (2003) In vitro study of the functional expression of organic anion transporting polypeptide 3 at rat choroid plexus epithelial cells and its involvement in the cerebrospinal fluid-to-blood transport of estrone-3-sulfate. *Mol Pharmacol* **63:**532-537.

Poirier A, Funk C, Lave T and Noe J (2007) New strategies to address drug-drug interactions involving OATPs. *Curr Opin Drug Discov Devel* **10:**74-83.

Polasek TM and Miners JO (2006) Quantitative prediction of macrolide drug-drug interaction potential from in vitro studies using testosterone as the human cytochrome P4503A substrate. *Eur J Clin Pharmacol* **62:**203-208.

Seithel A, Eberl S, Singer K, Auge D, Heinkele G, Wolf NB, Dorje F, Fromm MF and Konig J (2007) The influence of macrolide antibiotics on the uptake of organic anions and drugs mediated by OATP1B1 and OATP1B3. *Drug Metab Dispos* **35:**779-786.

Seithel A, Karlsson J, Hilgendorf C, Bjorquist A and Ungell AL (2006) Variability in mRNA expression of ABC- and SLC-transporters in human intestinal cells: comparison between human segments and Caco-2 cells. *Eur J Pharm Sci* **28:**291-299.

Shirasaka Y, Li Y, Shibue Y, Kuraoka E, Spahn-Langguth H, Kato Y, Langguth P and Tamai I (2009) Concentration-dependent effect of naringin on intestinal absorption of beta(1)-adrenoceptor antagonist talinolol mediated by p-glycoprotein and organic anion transporting polypeptide (Oatp). *Pharm Res* **26:**560-567.

Smith NF, Figg WD and Sparreboom A (2005) Role of the liver-specific transporters OATP1B1 and OATP1B3 in governing drug elimination. *Expert Opin Drug Metab Toxicol* **1:**429-445.

Tani T, Gram LK, Arakawa H, Kikuchi A, Chiba M, Ishii Y, Steffansen B and Tamai I (2008) Involvement of organic anion transporting polypeptide 1a5 (Oatp1a5) in the intestinal absorption of endothelin receptor antagonist in rats. *Pharm Res* **25:**1085-1091.

Vavricka SR, Van Montfoort J, Ha HR, Meier PJ and Fattinger K (2002) Interactions of rifamycin SV and rifampicin with organic anion uptake systems of human liver. *Hepatology* **36:**164-172.

Walters HC, Craddock AL, Fusegawa H, Willingham MC and Dawson PA (2000) Expression, transport properties, and chromosomal location of organic anion transporter subtype 3. *Am J Physiol Gastrointest Liver Physiol* **279:**G1188-1200.

Yarim M, Moro S, Huber R, Meier PJ, Kaseda C, Kashima T, Hagenbuch B and Folkers G (2005) Application of QSAR analysis to organic anion transporting polypeptide 1a5 (Oatp1a5) substrates. *Bioorg Med Chem* **13:**463-471.

Footnotes

This project was supported by a grant from GlaxoSmithKline. A.R. was supported by a National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases Research Service Award (F32 DK079576).

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legends

Figure 1. Inhibition of taurocholate uptake by macrolides in MDCK-rat Oatp1a5 cells. The inhibitory effect of (A) azithromycin, (B) clarithromycin, and (C) erythromycin on rat Oatp1a5-mediated [^{3}H]taurocholate (5 μM) uptake was measured. The Oatp1a5-specific uptake of taurocholate was determined by subtracting the [^{3}H]taurocholate uptake by parental MDCK cells incubated under parallel conditions as the MDCK-rat Oatp1a5 cells. Each data point represents the mean ± SEM of triplicate determinations. Taurocholate uptake in the absence of azithromycin (48.2 ± 11.4 pmol•mg cell protein^{-1}•10 min^{-1}), clarithromycin (52.1 ± 9.4 pmol•mg cell protein^{-1}•10 min^{-1}) or erythromycin (41.4 ± 11.7 pmol • mg cell protein^{-1} • 10 min^{-1}) was set at 100%. The EC_{50} values, the concentration of macrolide required to inhibit 50% of specific [^{3}H]taurocholate uptake, were calculated by fitting the data to a sigmoidal dose-response curve using GraphPad Prism.

Figure 2. Analysis of the inhibition of taurocholate uptake by macrolides in MDCK-rat Oatp1a5 cells. (A, B) The uptake of [^{3}H]taurocholate at the fixed concentrations of 5 μM, 10 μM, and 25 μM was measured in the absence or presence of the indicated concentrations of (A) azithromycin, or (B) clarithromycin. (C, D) Dixon plot analysis of taurocholate uptake data in the presence of (C) azithromycin, or (D) clarithromycin. Each point represents the mean ± SEM of triplicate determinations. The apparent K_i value, the concentration of macrolide required to double the slope of the 1/velocity versus 1/substrate concentration plot, was calculated from the fitted lines using GraphPad Prism. The K_i values for azithromycin and clarithromycin inhibition of Oatp1a5-mediated taurocholate uptake were 3.3 ± 0.2 μM and 2.4 ± 0.1 μM, respectively (mean ± SEM).

DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3. Inhibition of estrone-3-sulfate uptake by macrolides in human OATP1A2-transfected COS cells. COS cells were transiently transfected with human OATP1A2 plasmid or a mixture of control expression plasmids. The OATP1A2-specific uptake was determined by subtracting the [^{3}H]estrone-3-sulfate uptake for control plasmid-transfected COS cells incubated under parallel conditions. (A) The transfected COS cells were incubated for 10 min at 37°C in pH 6.0 buffer containing 5 µM [^{3}H]estrone-3-sulfate plus 250 µM of the indicated competitor, bromosulfophthalein (BSP), rifamycin SV (Rif), azithromycin (Azi), clarithromycin (Clari), or erythromycin (Ery). Estrone-3-sulfate uptake in the absence of competitor, 28.7 $\pm$ 2.1 pmol•mg cell protein^{-1}•10 min^{-1}, was set at 100%. (B to E) The inhibitory effect of (B) azithromycin, (C) clarithromycin, (D) erythromycin, and (E) BSP on human OATP1A2-mediated [^{3}H]estrone-3-sulfate (1 µM) uptake. Estrone-3-sulfate uptake in the absence of azithromycin (4.4 $\pm$ 0.1 pmol•mg cell protein^{-1}•10 min^{-1}), clarithromycin (2.7 $\pm$ 0.1 pmol•mg cell protein^{-1}•10 min^{-1}), erythromycin (9.3 $\pm$ 0.2 pmol•mg cell protein^{-1}•10 min^{-1}), or BSP (3.8 $\pm$ 0.2 pmol•mg cell protein^{-1}•10 min^{-1}) was set at 100%. Each bar or point represents the mean $\pm$ SEM of triplicate determinations. * $P < 0.05$; ** $P < 0.005$, versus uptake in the absence of competitor (control).

Figure 4. Uptake of radiolabeled solutes by rat Oatp1a5 and human OATP1A2. COS cells were transiently transfected with (A) rat Oatp1a5 or (B) human OATP1A2 and a mixture of control expression plasmids. The transfected COS cells were incubated for 10 min at 37°C in pH 6.0 buffer with the indicated concentrations of [^{3}H]azithromycin, [^{3}H]clarithromycin, or [^{3}H]estrone-3-sulfate, and then processed to determine cell-associated protein and radioactivity. Each bar represents the mean $\pm$ SEM (n = 6). ** $P < 0.005$, versus control plasmid-transfected cells (YFP).

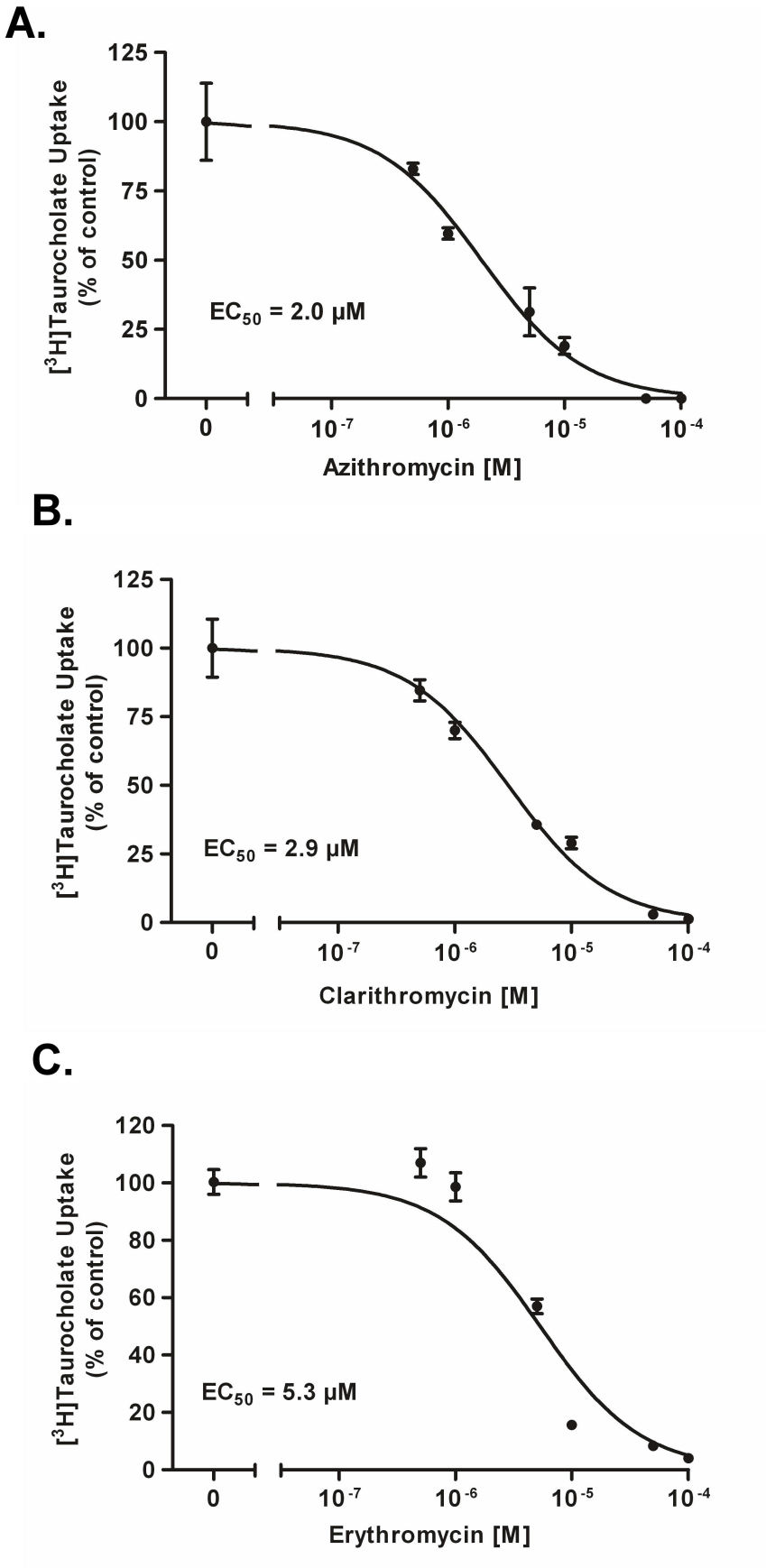
DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5. Inhibition of estrone-3-sulfate uptake by macrolides in rat Oatp2b1 and human OATP2B1-transfected COS cells. COS cells were transiently transfected with (A) rat Oatp2b1, (B) human OATP2B1, or a mixture of control expression plasmids. The transfected COS cells were incubated for 10 min in pH 6.0 buffer at 37°C with 5 µM [^{3}H]estrone-3-sulfate plus 250 µM of the indicated competitor. The Oatp2b1/OATP2B1-specific uptake was determined by subtracting the [^{3}H]estrone-3-sulfate uptake for control expression plasmid-transfected COS cells incubated under parallel conditions. Estrone-3-sulfate uptake in the absence of competitor, 5.8 $\pm$ 0.1 and 45.8 $\pm$ 0.6 pmol•mg cell protein^{-1}•10 min^{-1} for rat Oatp2b1 and human OATP2B1 respectively, was set at 100%. Each bar represents the mean $\pm$ SEM of triplicate determinations. ***P* < 0.005 versus uptake in the absence of competitor (control).

Figure 6. Inhibition of estrone-3-sulfate uptake by macrolides in human OATP2B1-transfected COS cells. The inhibitory effect of (A) azithromycin, (B) clarithromycin, (C) erythromycin, and (D) BSP on human OATP2B1-mediated uptake of 1 µM [^{3}H]estrone-3-sulfate. Estrone-3-sulfate uptake in the absence of azithromycin (15.0 $\pm$ 0.3 pmol•mg cell protein^{-1}•10 min^{-1}), clarithromycin (15.3 $\pm$ 0.6 pmol•mg cell protein^{-1}•10 min^{-1}), erythromycin (27.3 $\pm$ 0.7 pmol•mg cell protein^{-1}•10 min^{-1}), or BSP (15.1 $\pm$ 0.2 pmol•mg cell protein^{-1}•10 min^{-1}) was set at 100%. Each point represents the mean $\pm$ SEM of triplicate determinations.

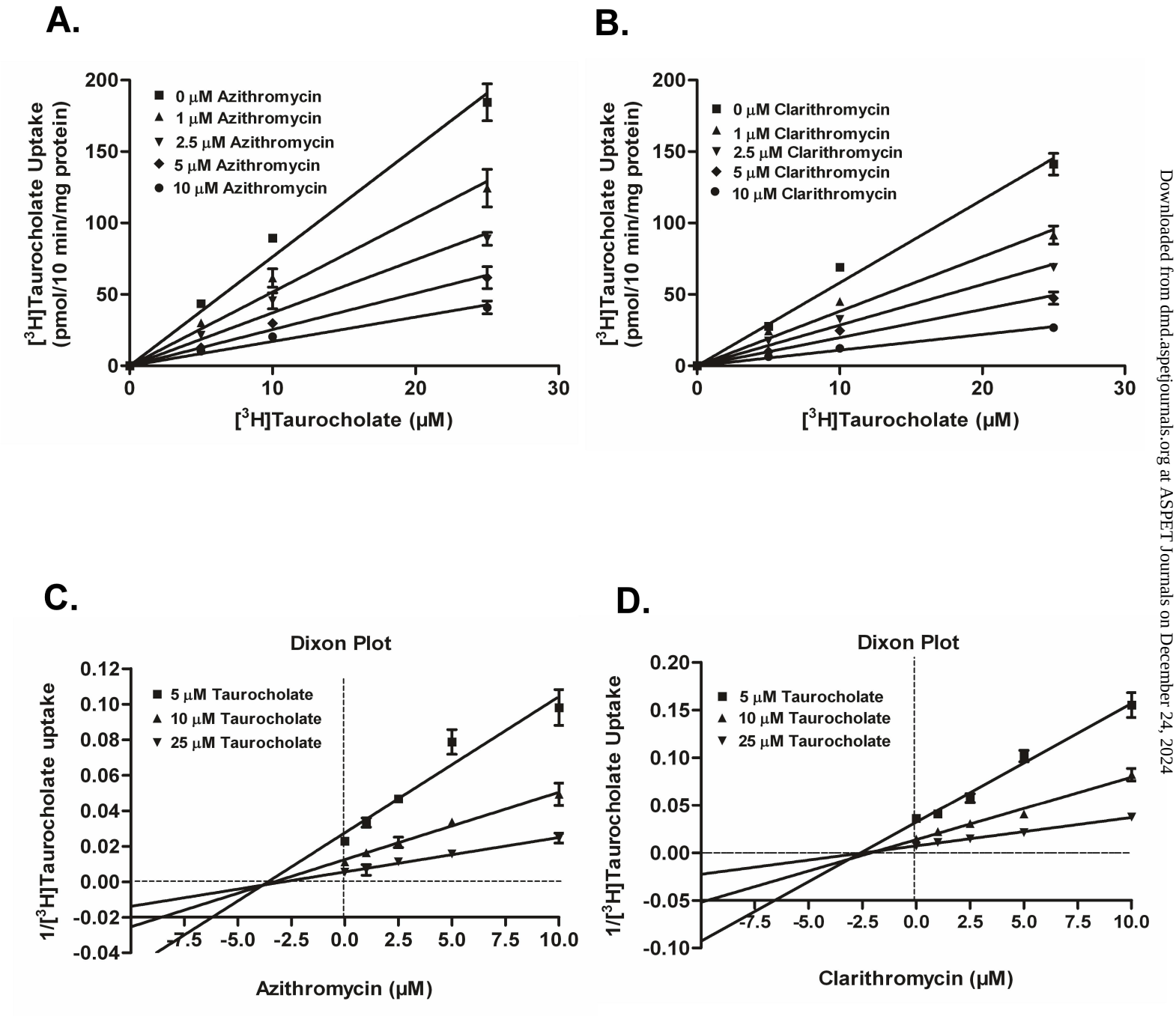
DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1



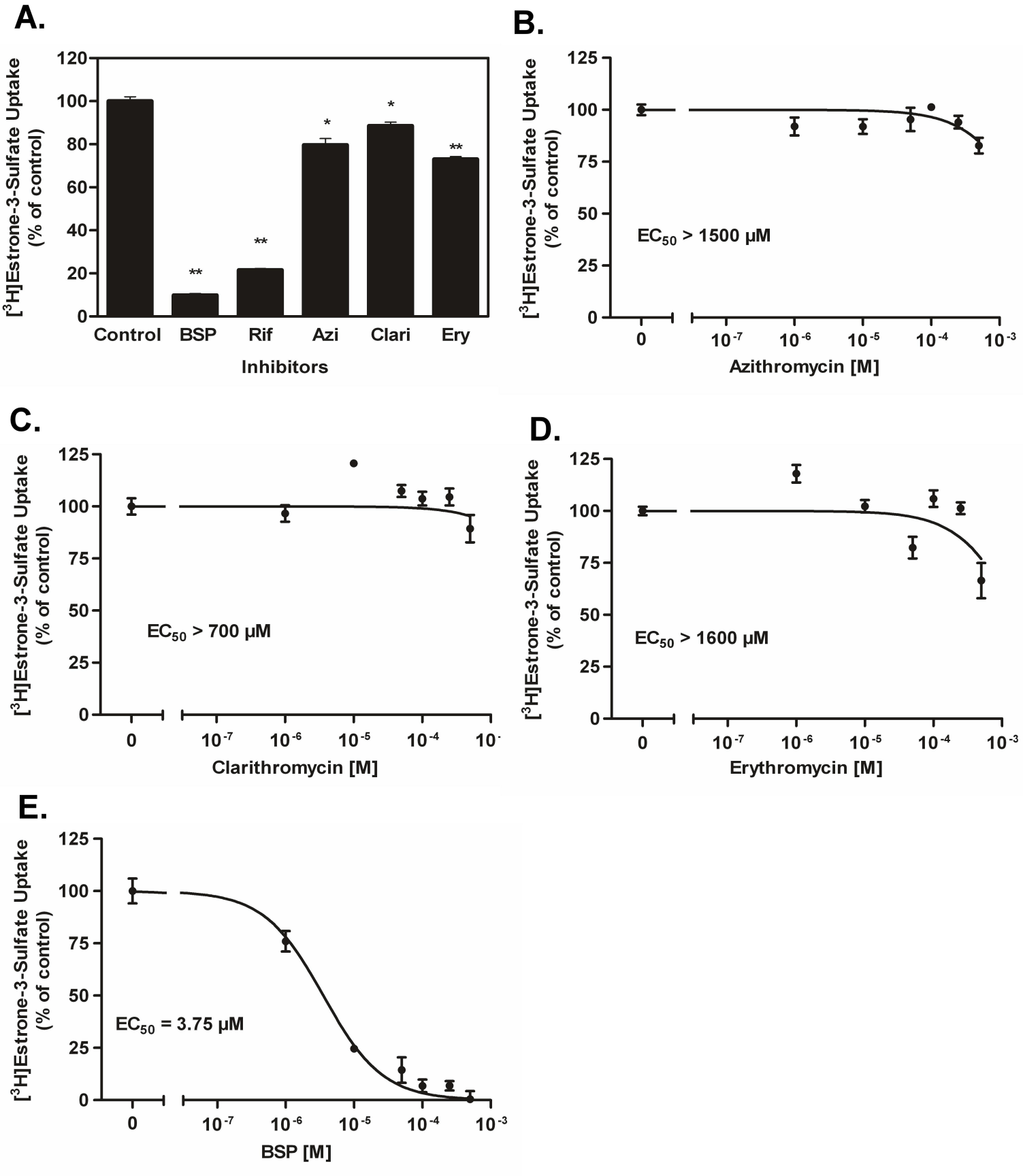
DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2



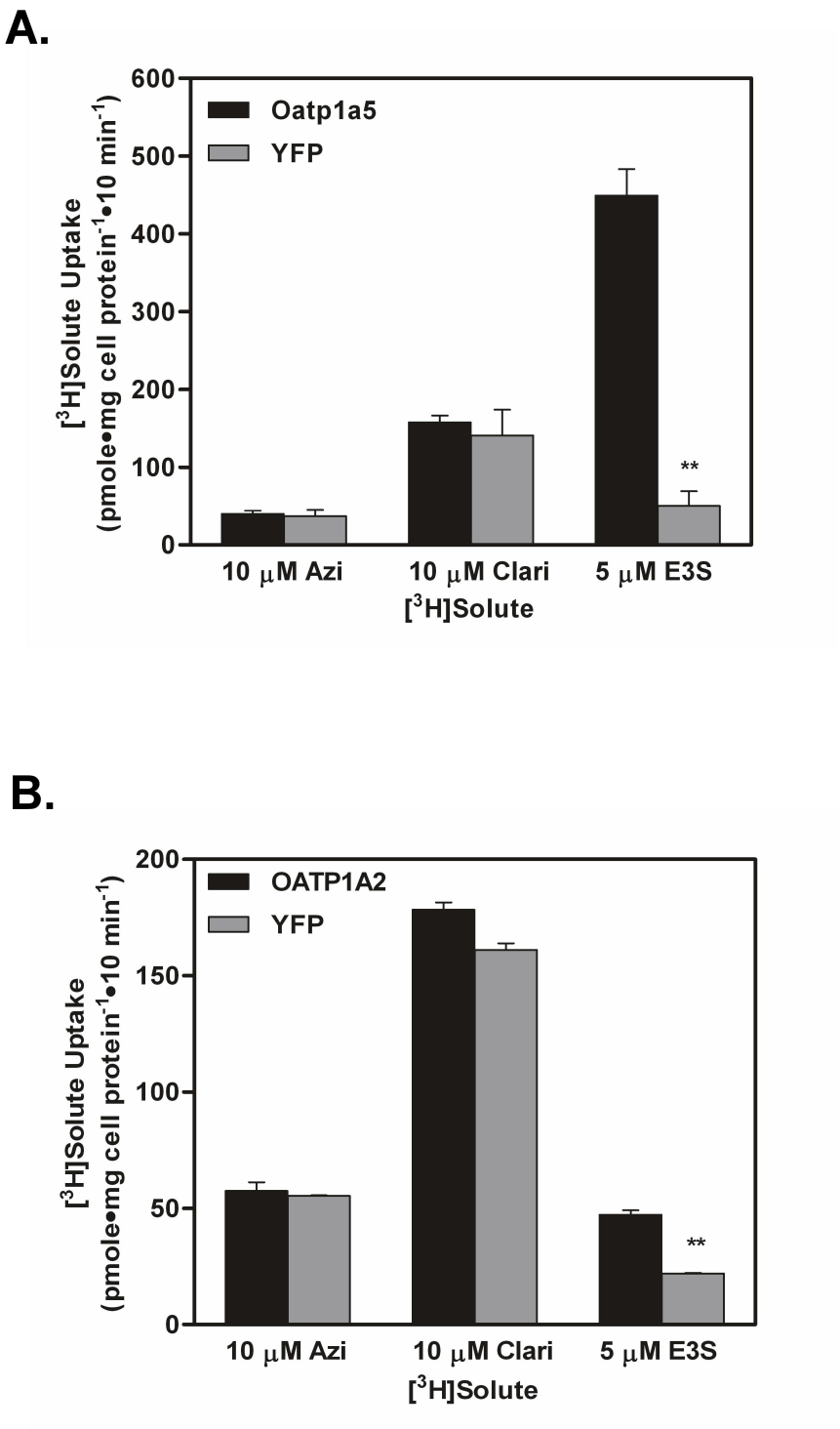
DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3



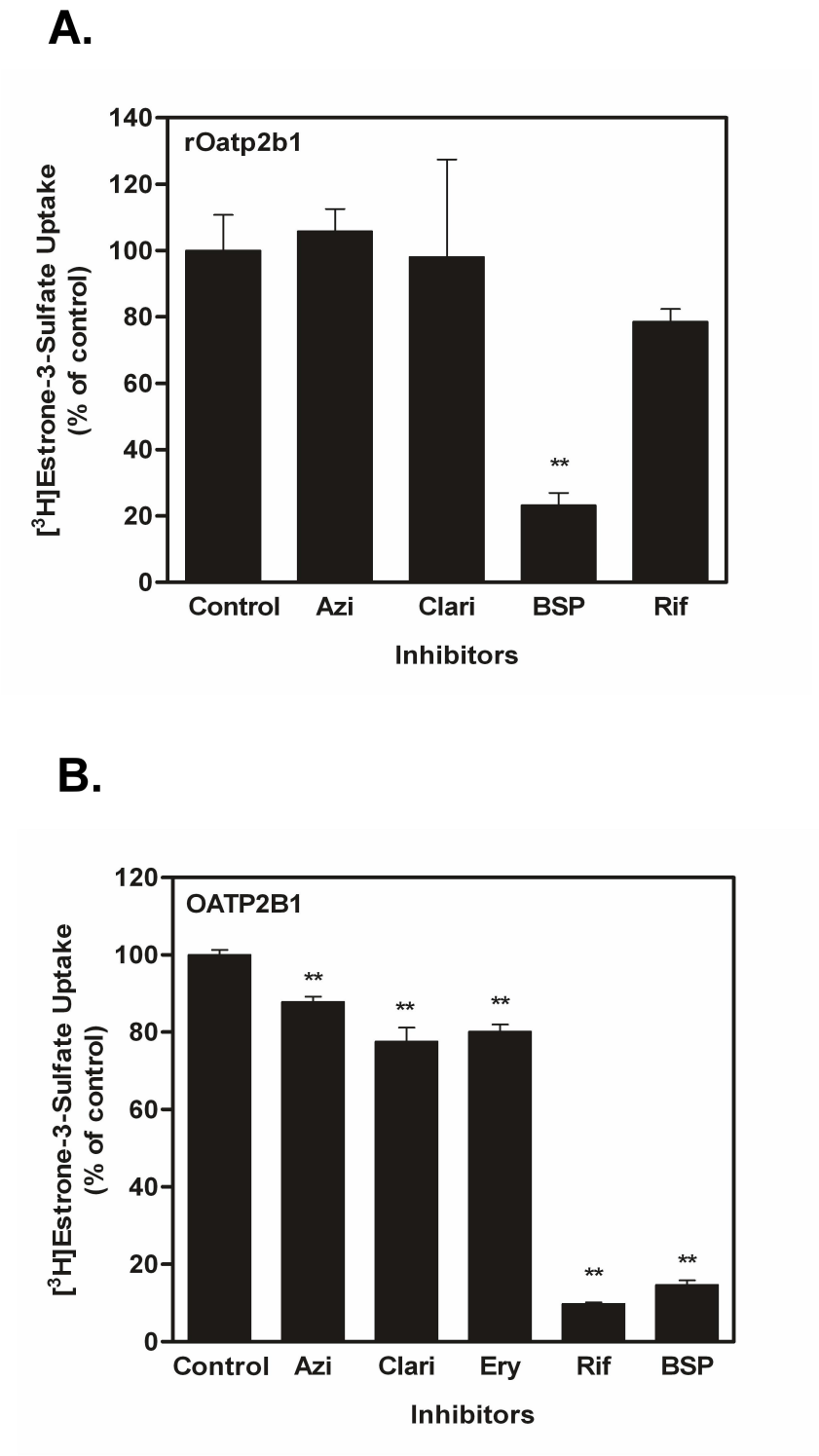
DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 4



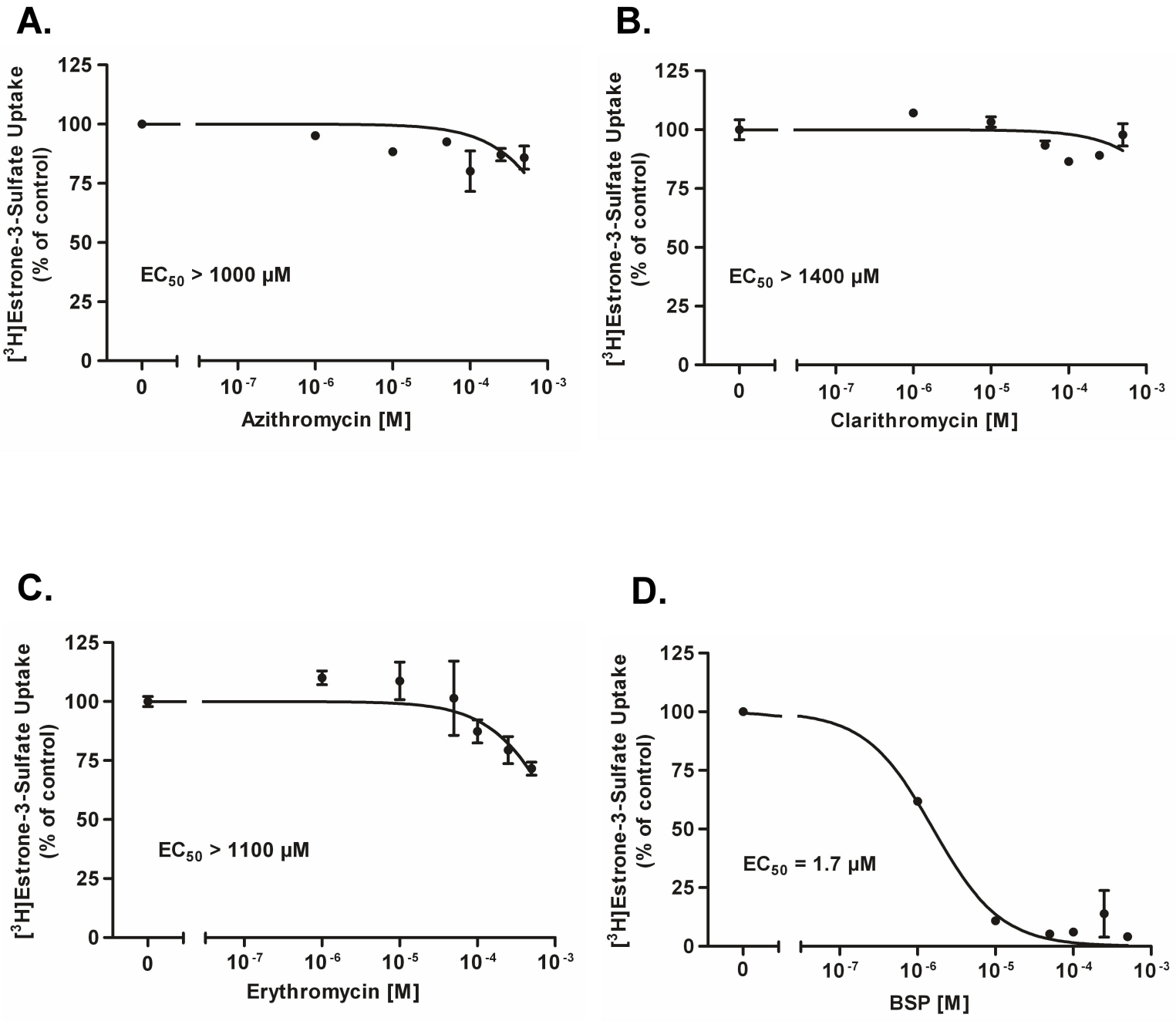
DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5



DMD Fast Forward. Published on September 9, 2009 as DOI: 10.1124/dmd.109.028522
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 6



Supplemental Data

Figure Legends

Figure 7. Effect of extracellular pH on solute transport by rat Oatp1a5, human OATP1A2, and human OATP2B1. COS cells were transiently transfected with (A, B) human OATP1A2, (C, D) rat Oatp1a5, (E, F) human OATP2B1 expression plasmid or a mixture of control plasmids. The transfected COS cells were pre-incubated for 30 min in uptake buffers at the indicated pH, and then incubated for 10 min in the same buffer plus 5 μM [^{3}H]taurocholate or [^{3}H]estrone-3-sulfate at 37°C. The cells were then processed to determine the cell-associated protein and radioactivity. Each bar represents the mean $\pm$ SEM of triplicate determinations. ** $P < 0.005$, versus control plasmids-transfected cells (YFP).

Figure 8. Uptake of radiolabeled solutes by MDCK-rat Oatp1a5 cells. MDCK-rat Oatp1a5 cells were incubated with (Oatp1a5) or without (control) sodium-butyrate to induce Oatp1a5 expression. After 20 h, the cells were washed and incubated in DMEM for 10 min at 37°C with the indicated concentrations of (A) [^{3}H]azithromycin, (B) [^{3}H]taurocholate, or (C) [^{3}H]clarithromycin and then processed to determine cell-associated radioactivity. Each bar represents the mean $\pm$ SEM of triplicate determinations. ** $P < 0.005$, versus uninduced cells.

Figure 9. Inhibition of estrone-3-sulfate uptake by macrolides in mouse Oatp2b1-transfected COS cells. COS cells were transiently transfected with mouse Oatp2b1 or control expression plasmid. The transfected COS cells were incubated for 10 min at 37°C with 5 μM [^{3}H]estrone-3-sulfate plus 250 μM of the indicated competitor. The Oatp2b1-specific uptake was determined by subtracting the [^{3}H]estrone-3-sulfate uptake for control expression plasmid-transfected COS cells incubated under parallel conditions. Estrone-3-sulfate uptake in the absence of competitor, 4.9 $\pm$ 0.1 pmol•mg cell protein^{-1}•10 min^{-1}, was set at 100%. Each bar

represents the mean ± SEM of triplicate determinations. ***P* < 0.005 versus cells incubated in the absence of competitor.

Figure 10. Uptake of radiolabeled solutes by human OATP2B1 and rat Oatp2b1. COS cells were transiently transfected with (A, B) human OATP2B1 or (C, D) rat Oatp2b1 expression plasmids. The transfected COS cells were incubated for 10 min at 37°C in pH 6.0 buffer with the indicated concentrations of [^{3}H]azithromycin, [^{3}H]clarithromycin, or [^{3}H]estrone-3-sulfate, and then processed to determine cell-associated protein and radioactivity. Each bar represents the mean ± SEM (n = 3). ** *P* < 0.005, versus control plasmid-transfected cells (YFP).

Figure 7

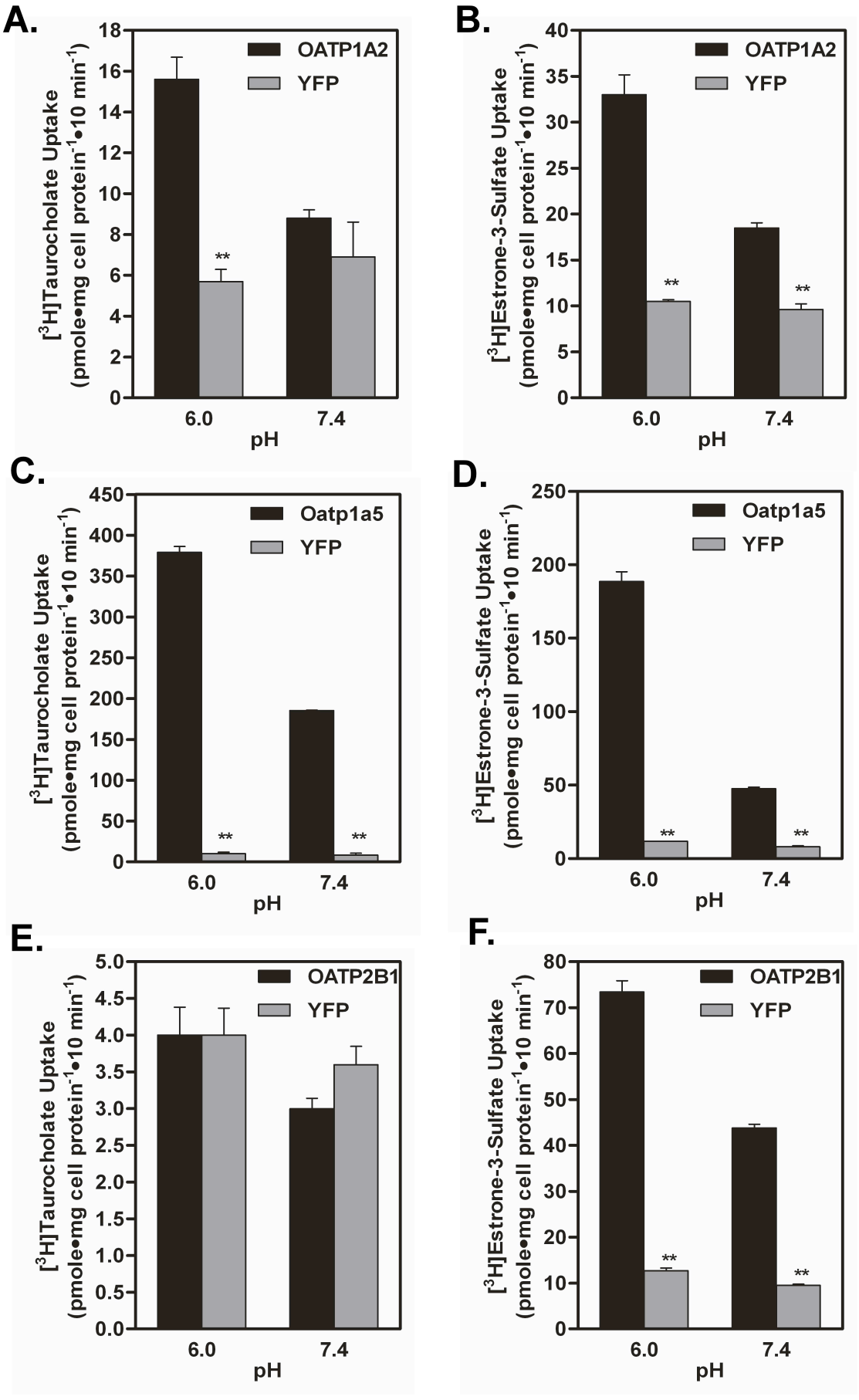

A.
B.
C.
D.
E.
F.
OATP1A2
YFP
Oatp1a5
OATP2B1
[3H]Taurocholate Uptake
(pmole•mg cell protein-1•10 min-1)
[3H]Estrone-3-Sulfate Uptake
(pmole•mg cell protein-1•10 min-1)
pH
6.0
7.4
**

Figure 8

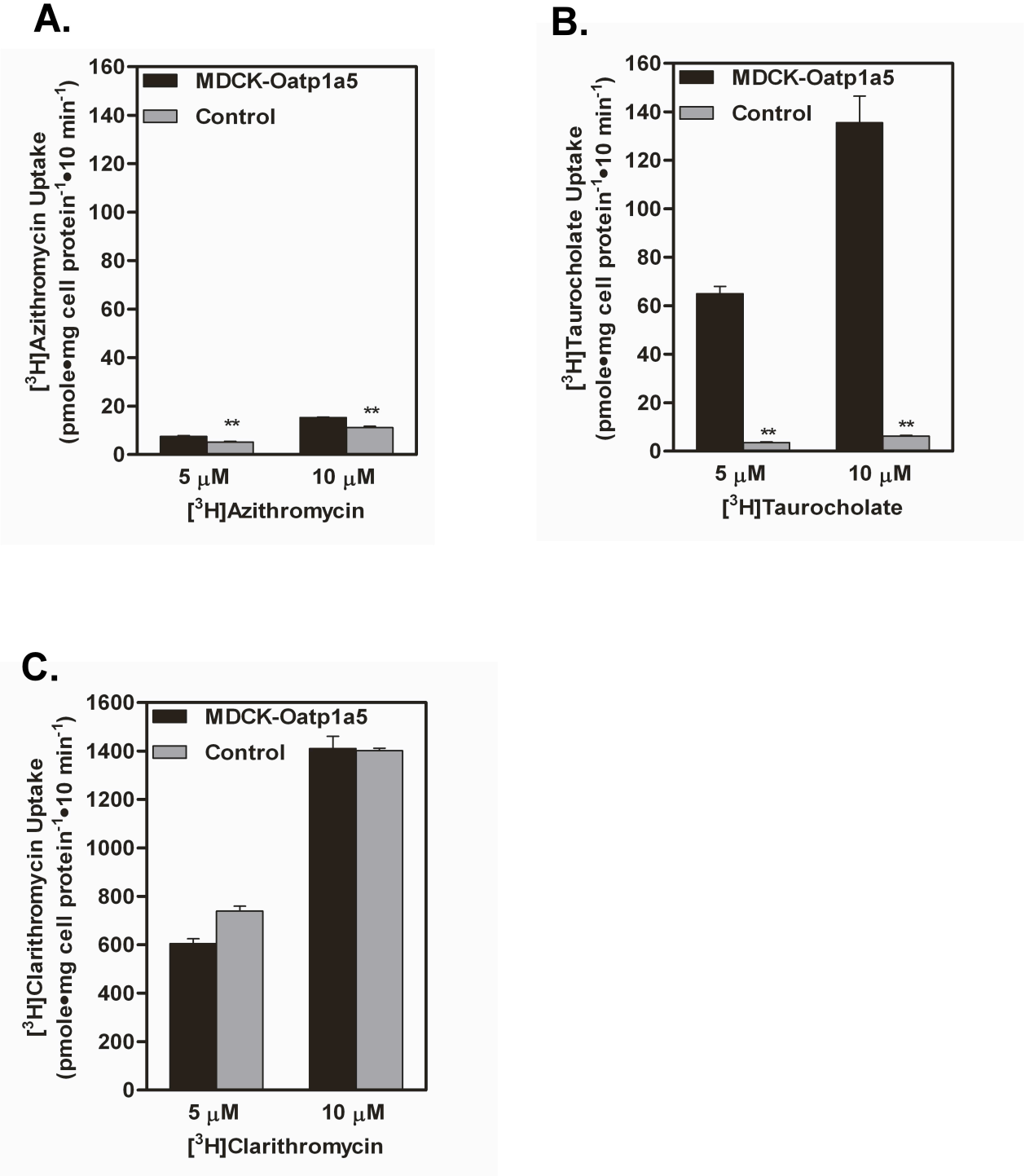

A.
MDCK-Oatp1a5
Control
[3H]Azithromycin Uptake
(pmole•mg cell protein-1•10 min-1)
160
140
120
100
80
60
40
20
0
**
**
5 μM
10 μM
[3H]Azithromycin
B.
MDCK-Oatp1a5
Control
[3H]Taurocholate Uptake
(pmole•mg cell protein-1•10 min-1)
160
140
120
100
80
60
40
20
0
**
**
5 μM
10 μM
[3H]Taurocholate
C.
MDCK-Oatp1a5
Control
[3H]Clarithromycin Uptake
(pmole•mg cell protein-1•10 min-1)
1600
1400
1200
1000
800
600
400
200
0
5 μM
10 μM
[3H]Clarithromycin

Figure 9

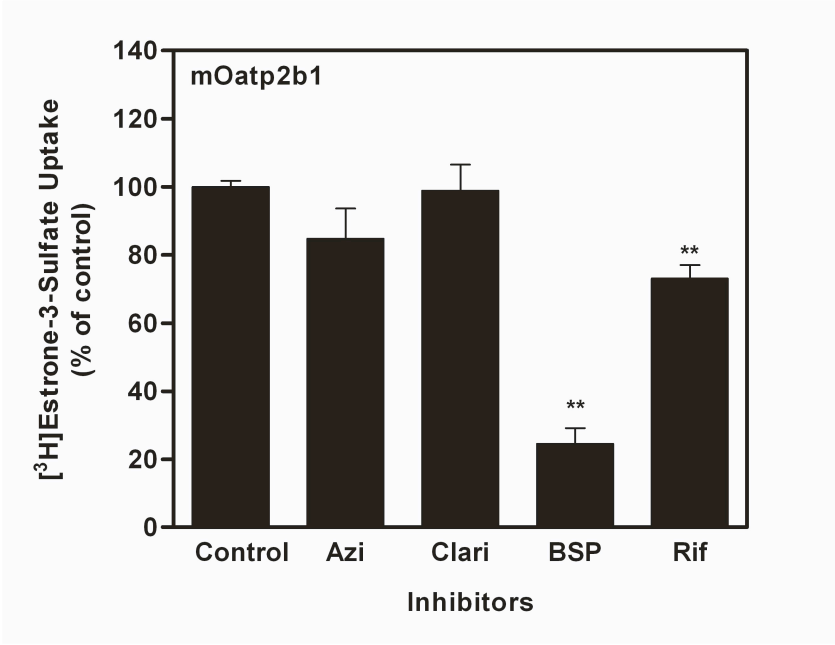
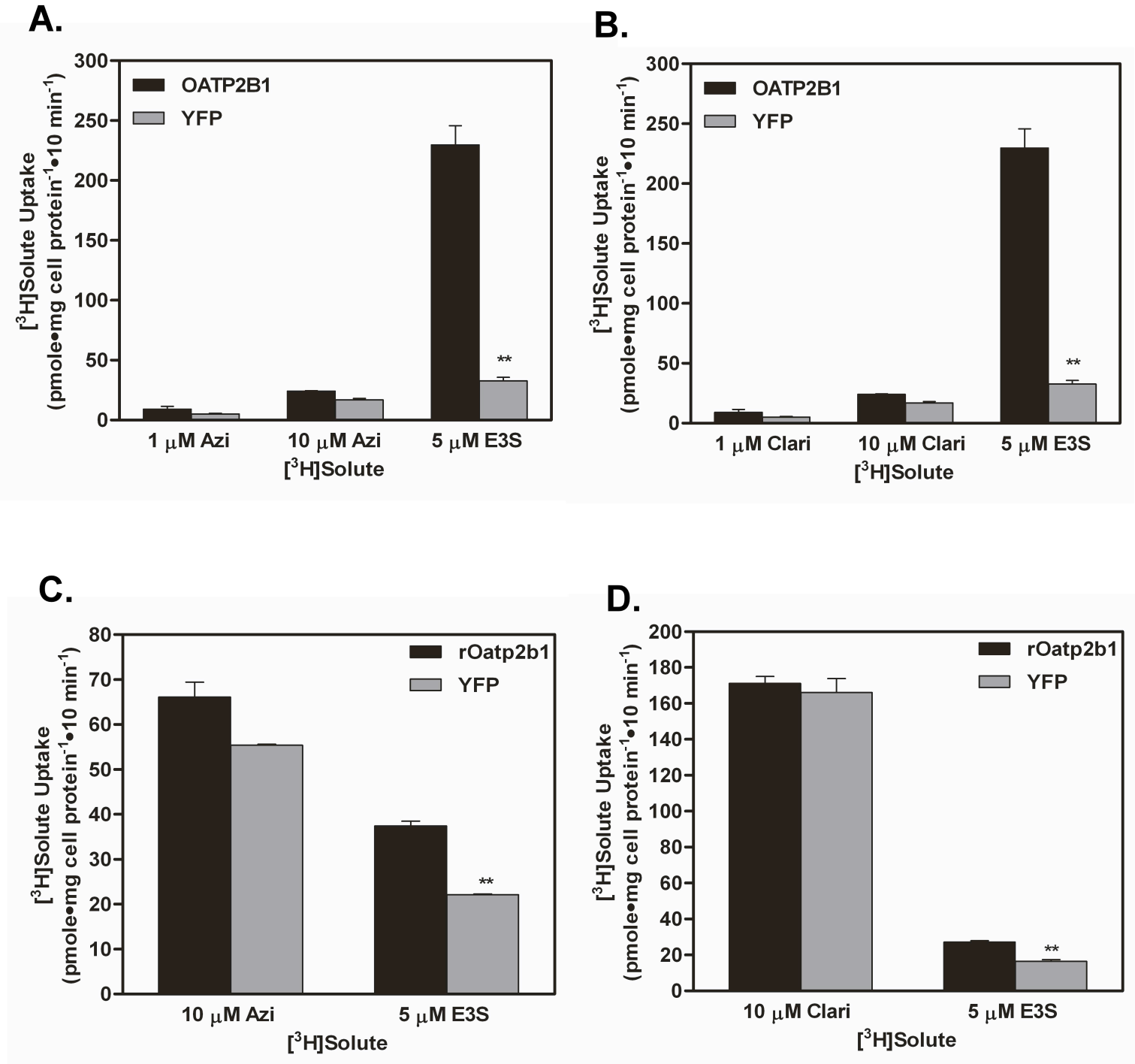

mOatp2b1
[3H]Estrone-3-Sulfate Uptake
(% of control)
140
120
100
80
60
40
20
0
**
**
Control
Azi
Clari
BSP
Rif
Inhibitors

Figure 10


A.
B.
C.
D.
OATP2B1
YFP
rOatp2b1
[3H]Solute Uptake
(pmole•mg cell protein-1•10 min-1)
1 μM Azi
10 μM Azi
5 μM E3S
1 μM Clari
10 μM Clari
[3H]Solute
**