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Impact on creatinine renal clearance by the interplay of multiple renal transporters – A Case Study with INCB039110

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Abbreviations:

GFR, glomerular filtration rate; JAK, Janus kinase; OCT, organic cation transporter; OAT, organic anion transporter; MATE, multidrug and toxin extrusion protein; MDCK-II, Madin-Darby Canine Kidney II; GMR, geometric mean ratio; GFP, Green Fluorescent Protein; IC_{50}, half-maximal inhibitory concentration.

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Abstract

Serum creatinine is commonly used as a marker of renal function but increases in serum creatinine may not represent changes in glomerular filtration rate (GFR). INCB039110 is an inhibitor of the Janus kinases (JAKs) with selectivity for JAK1. In a Phase I study, a modest and reversible increase in serum creatinine was observed following treatment with INCB039110. However, a dedicated renal function study with INCB039110 conducted in healthy volunteers indicated no change in GFR, assessed by iohexol plasma clearance. In vitro studies were therefore conducted to investigate the interaction of INCB039110 with five transporters that are likely involved in the renal clearance of creatinine. Cell systems expressing individual or multiple transporters were used including a novel quintuple-transporter model OAT2/OCT2/OCT3/MATE1/MATE2-K. INCB039110 potently inhibited OCT2-mediated uptake of creatinine as well as MATE1/MATE2-K-mediated efflux of creatinine. Given the interactions of INCB039110 with multiple transporters affecting creatinine uptake and efflux, an integrated system expressing all five transporters was sought and in that system, INCB039110 caused a dose-dependent decrease in transcellular transport of creatinine with weaker net inhibition compared to effects on individual transporters. In summary, a molecular mechanism for the increase in serum creatinine by INCB039110 has been established. These studies also underline the limitations of using serum creatinine as a marker of renal function.

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Introduction

Creatinine is a cyclic anhydride of creatine and an end product of muscle metabolism. Creatinine clearance based on serum creatinine is used to estimate glomerular filtration rate (GFR) and is the most commonly used clinical indicator of renal function (Perrone et al, 1992). While creatinine is predominantly eliminated by glomerular filtration, it also undergoes active tubular secretion, which can account for 10% to 40% of creatinine clearance (Levey et al., 1988). Although the mechanisms underlying the renal tubular transport of creatinine have not been fully elucidated, it is known that multiple transporters are involved. Renal tubular secretion of creatinine was reported to be driven by uptake *via* human organic cation transporter 2 (OCT2) (Urakami et al., 2004; Koteff et al., 2012), organic cation transporter 3 (OCT3) (Imamura et al, 2011) and organic anion transporter 2 (OAT2) (Lepist et al., 2014). In addition, multidrug and toxin extrusion protein 1 (MATE1) and 2-K (MATE2-K), expressed on the apical side of the renal tubular cells, have been shown to play a role in the tubular efflux of creatinine (Tanihara et al., 2007). Hence, drugs can interact with one or more of these transporters to cause non-pathological elevation of serum creatinine and such elevations have been reported with several drugs, e.g. cimetidine (Hilbrands et al., 1991), trimethoprim (Naderer et al., 1997), tofacitinib (Lawendy et al, 2009), and dolutegravir (Koteff et al., 2012). During the clinical development of INCB039110, a JAK1 inhibitor, transient and reversible increases of serum creatinine were observed and in vitro studies were conducted to understand the mechanisms involved, including experiments using a novel multi-transporter cell system and the results from those investigations are presented in this paper.

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Materials and Methods

Materials

[^{14}C]-creatinine was purchased from Moravek Biochemicals (Brea, CA). Cell culture media and buffer solution were purchased from Corning-Cellgro (Manassas, VA). Porous membrane insert plates were purchased from Millipore (Billerica, MA). INCB039110 and placebo tablets were supplied by Incyte Corporation (Wilmington, DE). Iohexol was purchased commercially as Omnipaque™ from GE Healthcare (Princeton, NJ). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO). The purified subclone of Madin-Darby Canine Kidney II cells (MDCK-II) used in these experiments was isolated at Optivia Biotechnology (Menlo Park, CA).

Clinical Study Design

A definitive renal function study using INCB039110 was conducted at Prism Research (St. Paul, MN) in full accordance with the Declaration of Helsinki; the Good Clinical Practice as required by and described in 21 Code of Federal Regulations (CFR) parts 50, 54, 56, 312 subpart D, and 314. This was a randomized, placebo-controlled, two-way crossover study in healthy adult subjects aged 18 to 55 years. A total of 24 healthy male adult subjects were randomized to two treatment sequences (12 subjects per sequence), either active treatment first followed by placebo or placebo first followed by active treatment. Subjects received 6 x 100 mg INCB039110 tablets or matching placebo along with a standardized medium-fat meal, twice daily for 8 days. Following a washout period of 13 days the alternate treatment was administered. Iohexol 350 mg/mL was administered as a 20 mL IV infusion over 15 minutes and was given 2 hours after the INCB039110 or placebo morning dose on Days -1 (baseline), 8, 21 (baseline), and 29. Serum concentrations of creatinine and plasma concentrations of INCB039110 and iohexol were

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evaluated in accordance with the schedule of assessments. Iohexol and INCB039110 were extracted *via* protein precipitation or liquid/liquid extraction, respectively, followed by LC/MS/MS analysis using Sciex API-4000 (AB Sciex LLC, Foster City, CA) and multiple reaction monitoring (MRM). Standard non-compartmental pharmacokinetic (PK) methods were used to analyze iohexol and INCB039110 plasma concentration using Phoenix WinNonlin *ver* 6.0 (Pharsight Corporation, Mountain View, CA). GFR at the end of treatment was measured by iohexol plasma clearance (CL) and was baseline corrected. The geometric mean ratio (GMR) for active vs placebo treatment and its 90% confidence interval (CI) were determined using a 2-way crossover linear mixed-effect analysis of variance (ANOVA) model.

Cellular Uptake Study in OAT2, OCT2, or OCT3 Transfected MDCK-II cells

Fully confluent MDCK-II cell monolayers were transfected with DNA plasmids encoding one of the three uptake transporters - OAT2 (SLC22A7), OCT2 (SLC22A2), or OCT3 (SLC22A3) or Green Fluorescent Protein (GFP) as a control, at a final concentration of 30 ng/µL (Optivia Biotechnology, Menlo Park, CA). The transiently transfected MDCK-II cells were incubated for approximately 48 hr to allow the cells to become polarized and transporters being appropriately localized. Pre-incubation was carried out with HBSS (37°C) in both apical and basolateral compartments for 15 minutes. Uptake was initiated by replacing the buffer with HBSS containing 100 µM ^{14}C-creatinine (specific activity 0.05 Ci/mmol) and either reference inhibitors or INCB039110 in the basolateral compartment, followed by a 5 minute incubation at 37°C. The reference inhibitors used were cimetidine (for OCT2), indomethacin (for OAT2), and quinidine (for OCT3). At the end of incubation, cells were washed with ice cold PBS and permeabilized by incubation with 50% acetonitrile in water. Radioactivity was counted on a Wallac 1450

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Microbeta (Perkin Elmer, Waltham, MA).

Trans-cellular Transport Study in a Dual or Quintuple Transporter Model

The dual-transporter model for MATE1 (SLC47A1) and MATE2-K (SLC47A2) was created by transfecting fully confluent MDCK-II cell monolayers with a DNA mixture containing the plasmids encoding MATE1 and MATE2-K at concentrations of 15 and 10 ng/µL, respectively. The quintuple-transporter model consists of OAT2, OCT2, and OCT3, expressed on the basolateral cell membrane, and MATE1 and MATE2-K, expressed on the apical side of the cells. This model was created using similar procedure as described above for the dual transporter system with transfection carried out using a DNA mixture containing the plasmids encoding OCT2, OAT2, OCT3, MATE1 and MATE2-K transporters at concentrations of 20, 20, 10, 15, 10 ng/µL, respectively. Approximately 48 hours after transfection, cells of both dual and quintuple models were washed with HBSS. A 30 minute pre-incubation at 37°C with HEPES buffered HBSS (HBSS-HEPES, pH 7.4) containing either reference inhibitors or INCB039110 in both the apical and basolateral compartments was carried out. The buffer in the apical compartment was then replaced with Bis-Tris buffered HBSS (HBSS-Bis-Tris, pH 6.0) containing either the reference inhibitor or INCB039110. The buffer in the basolateral compartment was replaced with HBSS-HEPES buffer (pH 7.4) containing 100 µM ^{14}C-creatinine with either reference inhibitors or INCB039110. Following the 60 minute incubation, aliquots of the apical chambers were sampled and radioactivity was counted on a Wallac 1450 Microbeta to measure transcellular flux.

Analysis of Data from Transport Studies

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Transport studies were conducted in triplicate in both transporter-expressing cells and in GFP control cells. The percent inhibition was calculated by dividing the net transporter-mediated transport of creatinine in the presence of inhibitor by the corresponding value for vehicle. The IC_{50} value was calculated using the following equation:

$$V = \frac{V_0}{1 + ([I]/IC_{50})^n}$$

where V_0 is the mean transporter-mediated flux or accumulation in the presence of vehicle (0.5% DMSO), V is the transporter-mediated flux or accumulation in the presence of inhibitor, [I] is the inhibitor concentration, and n is a Hill coefficient. Unlike the IC_{50} values determined for the individual uptake transporters, the IC_{50} values determined for basolateral to apical transport in the multiple transporter models reflects net inhibition.

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Results & Discussion

Inconsequential elevations in serum creatinine without an impairment of renal function have been reported as early as in the 1970s when patients were treated with trimethoprim and sulfamethoxazole (or BACTRIM™) (Berglund et al, 1975). Recent studies suggested that several transporters, such as OCT2 (Urakami et al., 2004; Koteff et al., 2012), OCT3 (Imamura et al, 2011), OAT2 (Lepist et al., 2014), MATE1 and MATE2-K (Tanihara et al., 2007), are likely involved in the movement of creatinine from the blood into the kidney proximal tubule cells, and then into the urine. Thus, there are multiple points of interaction that can lead to a change in serum creatinine. Although studies have demonstrated the importance of individual renal transporters in creatinine renal secretion using single transporter transfected cell lines, the combined net effect of these transporters on the renal tubular secretion of creatinine is best studied using a dynamic multi-transporter system where the interplay of these transporters can be assessed.

INCB039110 (2-(3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-1-(1-(3-fluoro-2-(trifluoromethyl)isonicotinoyl)piperidin-4-yl)azetidin-3-yl)acetonitrile) is a JAK1 inhibitor in clinical development for multiple oncology indications (structure shown in Figure 1 A). Multiple dose studies in healthy volunteers showed small, reversible and dose-dependent increases in serum creatinine. To understand any pathological impact from multiple dosing of INCB039110, a definitive renal function study was conducted in healthy adult volunteers using iohexol as a marker of glomerular filtration. The changes in mean serum creatinine following multiple oral dosing of INCB039110 in that study are shown in Figure 1 B. The highest mean serum creatinine change of 19 µM was noted on the ninth day of INCB039110 treatment, compared with 6 µM for

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placebo, resulting in a 13 µM net increase of serum creatinine with INCB039110 treatment. At this time point, 2 subjects treated with INCB039110 had serum creatinine values above the upper limit of normal (ULN) although the mean values were within the normal range. At the follow-up visit, all INCB039110 subjects had serum creatinine measurements within the normal range, consistent with the findings from an earlier clinical study. The impact on renal function was then assessed by change in iohexol clearance following INCB039110 treatment compared to placebo treatment. The mean iohexol CL values were 6.99 L/h and 7.00 L/h at the end of treatment in the INCB039110 and placebo groups, respectively, on Day 8 and 6.99 L/h for both groups on Day 29. The plasma concentration vs time curves were superimposable on Days -1, 8, 21, and 29 (Figure 1C). The geometric mean ratio (GMR) of baseline-corrected GFR was 1.00 with a 90% CI of 0.956 - 1.05, i.e., INCB039110 was not significantly different from placebo. These results clearly indicate that INCB039110 treatment does not affect renal function and therefore the small increases in serum creatinine were likely resulting from modulation of transporters involved in creatinine disposition.

To understand the potential mechanism behind the increase in serum creatinine observed with INCB039110 treatment, the interaction of INCB039110 with the five known transporters involved in the uptake or efflux of creatinine was evaluated, using in vitro systems expressing a single or multiple transporters. In the case of single transporter systems, the functional activity of each transporter transfected into MDCK-II cells, viz, OCT2, OCT3 and OAT2, was confirmed by comparing the accumulation of probe substrates in the transfected cells vs GFP transfected mock control. Compared to the mock control cells, varying degrees of creatinine accumulation was observed in transporter transfected cells, 7.2-fold in the case of OAT2, 2.4-fold with OCT3

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and 2.2-fold with OCT2 (data on file). Addition of positive control inhibitors was shown to decrease transport of creatinine into these cells, confirming transporter mediated uptake of creatinine in the cell systems used. The basolateral uptake of creatinine mediated by OCT2, OCT3, and OAT2 was inhibited in the presence of various concentrations of INCB039110 in a concentration-dependent manner. The corresponding inhibition IC_{50} values were 5.8 µM, 84 µM and 80 µM by OCT2, OAT2, and OCT3, respectively (Fig 2A and Table 1).

INCB039110 also exhibited an inhibitory effect on the efflux of creatinine in the dual MATE1/MATE2-K co-expressing MDCK-II cells with an IC_{50} of 3.7 µM (Figure 2B and Table 1). Thus, INCB039110 can simultaneously inhibit both uptake and efflux of creatinine and therefore an integrated model that captures the interaction with all five transporters was sought. It was indeed feasible to transfect MDCK-II monolayers with all five transporters and is herein referred to as the quintuple model. The relative amounts of DNAs used in transfection were aimed to approximate the relative expression levels of the transporters in human kidney (Nishimura and Naito, 2005; Cheng et al, 2012; Morrissey et al, 2012). There are conflicting reports on the relative mRNA levels of OAT2 and OCT2 and therefore these two transporters were expressed at similar levels in the quintuple transporter model. INCB039110 exhibited a dose-dependent decrease in the transcellular transport of creatinine in the quintuple model with a maximum inhibition of 87% at 100 µM and an IC_{50} of 27 µM (Figure 2C and Table 1).

Next, an attempt was made to correlate the in vitro inhibitory data to observed in vivo changes in serum creatinine when treated with such inhibitors. Based on the observed mean unbound steady state plasma concentration of INCB039110 (~1 µM between 3h and 4h post-dose) and its inhibitory potency for creatinine transport in the quintuple transporter model, only modest

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impact on creatinine secretion by INCB039110 is expected, which is consistent with the small increases (~20%) observed in serum creatinine in the clinical study involving multiple doses of INCB039110. Drugs known to interact with creatinine secretion clinically *via* inhibition of transport (cimetidine and trimethoprim) were studied in this integrated cell system and compared to a negative control (salicylic acid). Cimetidine is a well-known competitive inhibitor of OCT2 (Imamura et al, 2011) and MATE1 (Matsushima et al 2009) transporters. In single transporter transfected cell lines, cimetidine inhibited all five transporters with varying inhibition potencies ranging all the way from 1.5 µM (potent) to 140 µM (weak) (Lepist et al., 2014). In the current study using the quintuple transporter model, the IC_{50} for cimetidine was 32 µM (Figure 2C and Table 2). Clinical studies have shown up to a 33 µM increase in serum creatinine after oral dosing of cimetidine (Hilbrands et al. 1991). In the case of trimethoprim, the observed IC_{50} value for trimethoprim in the quintuple cell model was 80 µM (Figure 2C and Table 2) and in a clinical study, an 18 µM increase in serum creatinine was observed (Berglund et al, 1975). It has been reported that the *in vitro* inhibition of creatinine secretion by trimethoprim may be primarily through blocking apical efflux mediated by MATE1 and MATE2 (Lepist et al., 2014). As a negative control, salicylic acid, which is known to elevate serum creatinine levels through glomerular damage (Caspi et al, 2000), did not inhibit creatinine transport in the quintuple model (Figure 2C). To compare the *in vitro* data with changes in observed serum creatinine levels, the ratios of unbound steady state plasma concentration, [I], and *in vitro* IC_{50} values obtained in the quintuple model, $[I]/IC_{50}$, of cimetidine, trimethoprim, and INCB039110 were calculated, as well as the relative $[I]/IC_{50}$ normalized to that of cimetidine (Table 2). While the dataset is limited, an apparent rank order exists among these three agents, suggesting that an in vitro – in vivo correlation may be possible using this multi-transporter model as more data are generated with

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additional agents that interact with these transporters. In particular, the availability of more potent inhibitors would be important in establishing robust correlations, which in turn may alleviate the need for dedicated renal function studies for some compounds.

In conclusion, small increases in serum creatinine were seen with the treatment of INCB039110; however, it did not affect the clearance of iohexol, a marker of glomerular filtration, in a dedicated renal function study. A molecular basis for the transient serum creatinine elevation observed after INCB039110 treatment is proposed that involves multiple transporters which play a role in both uptake and efflux of creatinine. The results from these studies demonstrate the utility of a cell system expressing all five transporters in quantitating the impact on creatinine clearance. Further, the conclusions drawn from the use of serum creatinine as a marker of renal function should be made with caution, acknowledging the possibility of artifactual increases through modulation of transporters involved in its clearance.

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Authorship Contributions

Participated in research design: Y Zhang, Warren, X Zhang, Diamond, Williams, Punwani, Y Huang, and Yeleswaram

Conducted experiments: X Zhang, Warren, Williams, and Punwani

Performed data analysis: Y Zhang, X Zhang, Warren, Williams, and Punwani

Wrote or contributed to the writing of the manuscript: Y Zhang, Warren, X Zhang, Diamond, Williams, J Huang, Y Huang, and Yeleswaram

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Reference

Berglund F, Killander J, and Pompeius R (1975) Effect of trimethoprim-sulfamethoxazole on the renal excretion of creatinine in man. *J Urol* 114: 802-808.

Caspi D, Lubart E, Graff E, Habot B, Yaron M, and Segal R. (2000) The effect of mini-dose aspirin on renal function and uric acid handling in elderly patients. *Arthritis Rheum* 43: 103-108.

Cheng Y, Vapurcuyan A, Shahidullah M, Aleksunes LM, and Pelis RM. (2012) Expression of organic anion transporter 2 in the human kidney and its potential role in the tubular secretion of guanine-containing antiviral drugs. *Drug Metab Dispos* 40: 617-624.

Hilbrands LB, Artz MA, Wetzels JF, and Koene RA (1991) Cimetidine improves the reliability of creatinine as a marker of glomerular filtration. *Kidney Int* 40: 1171-1176.

Imamura Y, Murayama N, Okudaira N, Kurihara A, Okazaki O, Izumi T, Inoue K, Yuasa H, Kusuhara H, and Sugiyama Y. (2011) Prediction of fluoroquinolone-induced elevation in serum creatinine levels: a case of drug-endogenous substance interaction involving the inhibition of renal secretion. *Clin Pharmacol Ther* 89: 81-88.

Koteff J, Borland J, Chen S, Song I, Peppercorn A, Koshiba T, Cannon C, Muster H, and Piscitelli SC (2012) A phase 1 study to evaluate the effect of dolutegravir on renal function via measurement of iohexol and para-aminohippurate clearance in healthy subjects. *Br J Clin Pharmacol* 75: 990-996.

Lawendy N, Krishnaswami S, Wang R, Gruben D, Cannon C, Swan S, and Chan G. (2009) Effect of CP-690,550, an orally active janus kinase inhibitor, on renal function in healthy adult volunteers. *J Clin Pharmacol* 49:423-429.

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DMD Fast Forward. Published on January 20, 2015 as DOI: 10.1124/dmd.114.060673
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Lepist EI, Zhang X, Hao J, Huang J, Kosaka A, Birkus G, Murray BP, Bannister R, Cihlar T, Huang Y, and Ray AS (2014) Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat. *Kidney Int* doi: 10.1038/ki.2014.66

Levey AS, Perrone RD, and Madias NE (1988) Serum creatinine and renal function. *Annu Rev Med* 39: 465-490.

Matsushima S, Maeda K, Inoue K, Ohta KY, Yuasa H, Kondo T, Nakayama H, Horita S, Kusuhara H, and Sugiyama Y. (2009) The inhibition of human multidrug and toxin extrusion 1 is involved in the drug-drug interaction caused by cimetidine. *Drug Metab. Dispos*. 37, 555–559.

Morrissey KM, Wen CC, Johns SJ, Zhang L, Huang SM, and Giacomini KM. (2012) The UCSF-FDA TransPortal: a public drug transporter database. *Clin Pharmacol Ther* 5:545-546.

Naderer O, Nafziger AN, and Bertino JS Jr (1997) Effects of moderate-dose versus high-dose trimethoprim on serum creatinine and creatinine clearance and adverse reactions. *Antimicrob Agents Chemother* 41: 2466-2470.

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.

Perrone RD, Madias NE, and Levey AS (1992) Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 38:1933-1953.

Somogyi A and Gugler R. (1983) Clinical pharmacokinetics of cimetidine. *Clin Pharmacokinet* 8: 463-495.

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This article has not been copyedited and formatted. The final version may differ from this version.

Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, and Inui K (2007) Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. *Biochem Pharmacol* 74: 359-371.

Urakami Y, Kimura N, Okuda M, and Inui K (2004) Creatinine transport by basolateral organic cation transporter hOCT2 in the human kidney. *Pharm Res* 21: 976-981.

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Footnote

Y Zhang, Diamond, Williams, Punwani, and Yeleswaram are employed by and are shareholders of Incyte Corporation. Warren, X Zhang, J Huang, and Y Huang are employed by Optivia Biotechnology, a contract service provider of transporter-related assays.

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Legends for Figures

Fig 1

A Chemical structure of INCB039110

B Mean change of serum creatinine by study day following 600 mg twice daily (BID) dose of INCB039110 for 8 days. Twenty-four healthy male adult subjects were randomized to 1 of 2 treatment sequences (12 subjects per sequence) that included 2 consecutive treatment periods separated by a 13-day washout period. Data represent the mean and standard error of 18 to 24 subjects that were on file at each time point. The baseline serum creatinine for all 24 subjects was 85 ± 9 μM (Mean ± SE). *, $p < 0.05$ versus placebo.

C Iohexol plasma concentration-time profiles in the subjects who completed the study (N = 17, Mean ± SE). One subject receiving INCB039110 treatment in Period 1 had an unusually high GFR value on Day 21. Statistically, this abnormally high GFR value was > 3× SD greater than the group mean of the 17 subjects on Day 21 and outside of the whisker of the box plot (data not shown). Therefore, it was treated as a statistical outlier and excluded from the final statistical analysis.

Fig. 2

A Effects of INCB039110 on the basolateral uptake of creatinine mediated by OCT2 (purple), OCT3 (blue) or OAT2 (orange). INCB039110 was studied in the concentration range of 0.1 – 300 μM. The percent inhibition (% of control) was calculated by dividing the net transporter-mediated transport of creatinine in the presence of INCB039110 by the net transporter-mediated transport of creatinine in the presence of vehicle. The IC_{50} value was determined by a non-linear regression using GraphPad Prism. Data represent the mean and standard deviation of triplicate samples.

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B Inhibition of efflux of creatinine by INCB039110 in the dual MATE1/MATE2-K model. INCB039110 was studied in the concentration range of 0.1 – 100 µM. The percent inhibition (% of control) was calculated by dividing the net MATE1/MATE2-K-mediated transport of creatinine in the presence of INCB039110 by the net MATE1/MATE2-K-mediated transport of creatinine in the presence of vehicle. The IC_{50} value was determined by a non-linear regression using GraphPad Prism. Data represent the mean and standard deviation of triplicate samples.

C Inhibition of transcellular creatinine transport in the quintuple OAT2/OCT2/OCT3/MATE1/MATE2-K model. The IC_{50} values were determined by non-linear regression using GraphPad Prism and were 32 µM for cimetidine (orange), 80 µM for trimethoprim (blue), 27 µM for INCB039110 (purple), and not calculable due to insufficient inhibition for salicylic acid (green). Data represent the mean and standard deviation of triplicate samples.

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Table 1. Inhibition of Creatinine Transport by INCB039110 or by a reference inhibitor

(IC_{50} values in μM, mean ± SD; N=3)

Transporter	INCB039110	Reference Inhibitor	
OAT2	84 ± 30	**Indomethacin**	6.5 ± 1.1
OCT2	5.8 ± 2.0	**Cimetidine**	17 ± 3.3
OCT3	80 ± 28	**Quinidine**	150 ± 39
MATE1/MATE2-K	3.7 ± 1.9	**Cimetidine**	4.6 ± 2.2
OAT2/OCT2/OCT3/MATE1/MATE2-K	27 ± 7.0	**Cimetidine**	32 ± 7.1

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Table 2. Comparison between the in vitro creatinine transport and in vivo serum creatinine elevation upon inhibition of renal transporters

Compound	Total [I][a] (μM)	Unbound [I] (μM)	Quintuple Model IC_{50} (μM)	Unbound $[I]/IC_{50}$	Normalized $[I]/IC_{50}$ (to Cimetidine)	Serum Creatinine[b] (μM)	Ref
INCB039110	~ 3	~ 1	27	0.037	0.37	13	Incyte unpublished observation
Trimethoprim	5.9	3.3	80	0.041	0.41	18	Berglund et al, 1975; FDA BACTRIM drug label
Cimetidine	4	3.2	32	0.10	1.0	33	Hilbrands et al. 1991; Somogyi and Gugler, 1983

[a][I], mean total steady state plasma concentration

[b], mean placebo adjusted change

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Figure 1

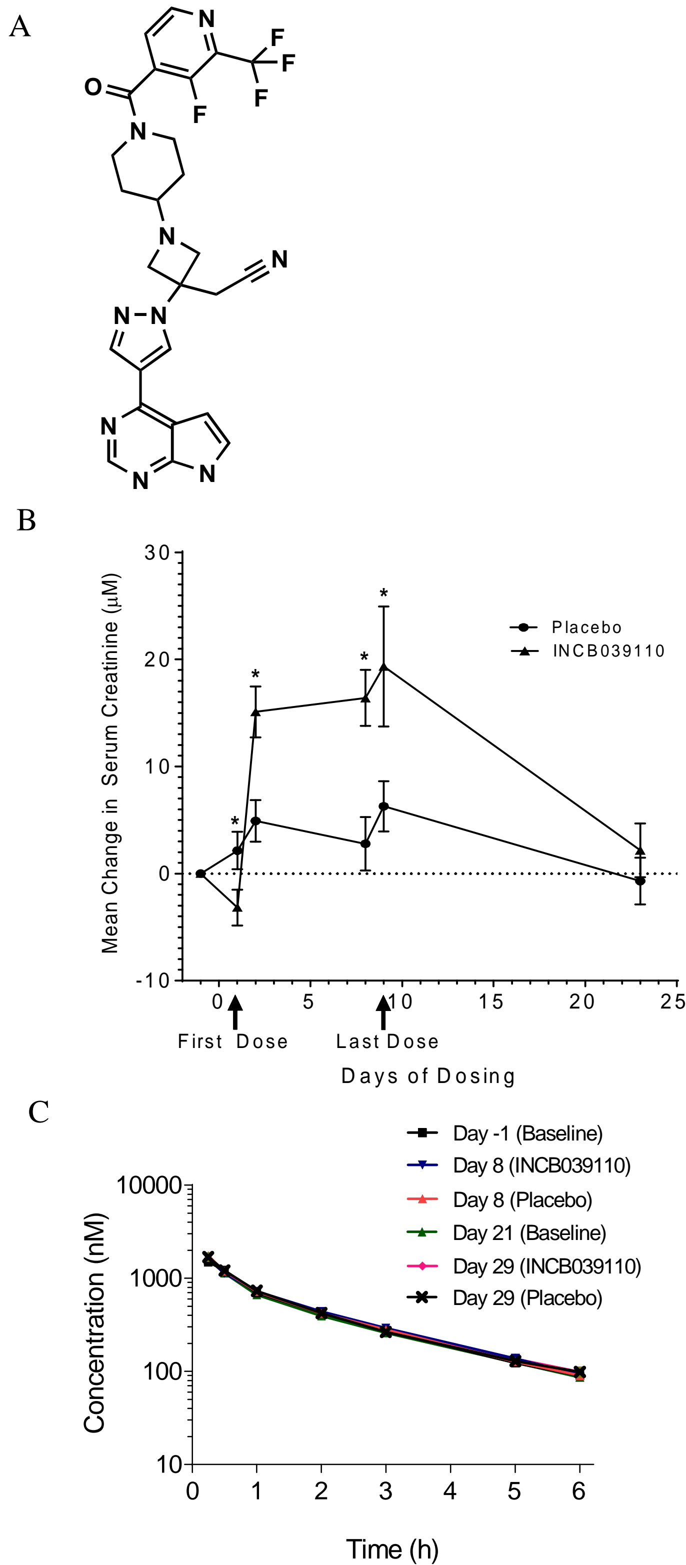

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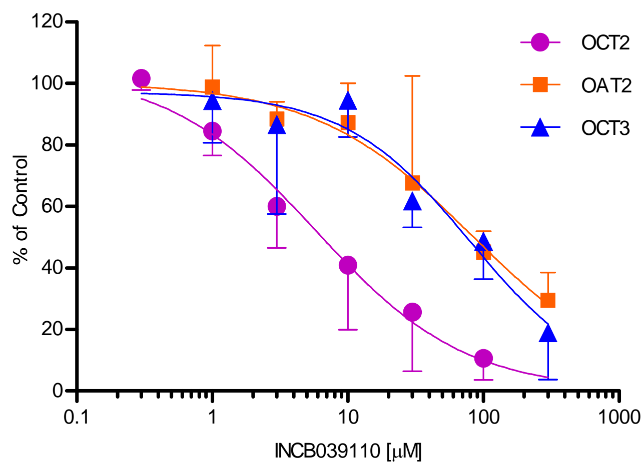
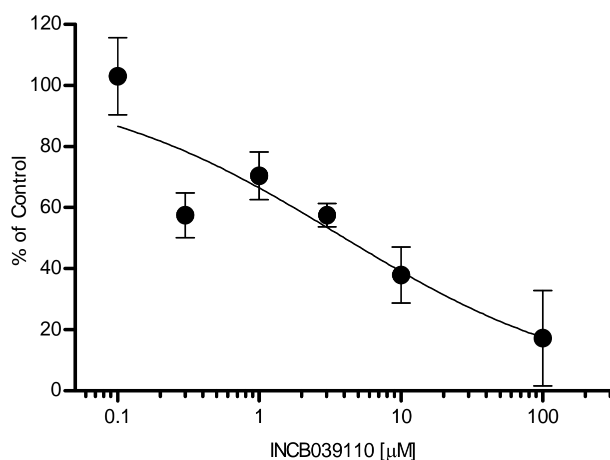
Figure 2

A



B



C