# Brilaroxazine Displays Limited Interaction with Clinically Relevant Drug Transporters

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## BACKGROUND

Brilaroxazine, a novel multimodal neuromodulator, belongs to a class of next-generation treatments for schizophrenia and comorbid conditions.[1-2] This novel multimodal dopamine (D) and serotonin (5-HT) receptor neuromodulator mitigates multiple inflammatory cytokines.[1-9] It specifically functions as a $D_{2/3/4}$ and 5-$HT_{1A/2A}$ partial agonist and a 5-$HT_{2B/7}$ antagonist with binding affinity for 5-$HT_{2B}$ > $D_2$.[1-4]

This agent possesses an established efficacy, safety, and pharmacokinetic (PK) profile based on its Phase 1 and 2 clinical experience, including the Phase 2 REFRESH study.[1,2,10] It offers a differentiated pharmacological and safety profile over other antipsychotics.[1-10] Brilaroxazine's Phase 3 RECOVER study reflects a highly effective and well-tolerated treatment at the 50 mg daily dose and neuroinflammatory capabilities.[8,9]

Transporters influence drug absorption, distribution, and elimination, leading to PK and pharmacodynamic drug-drug interactions (DDIs) with co-administered inhibitors and/or inducers. Conversely, a drug can also modulate transporter functions, resulting in altered disposition of endogenous and exogenous substances. Regulatory agencies have recommended that sponsors should evaluate their investigational drugs both as substrates and inhibitors of clinically relevant transporters.

## OBJECTIVE

This *in vitro* study's primary focus is to evaluate brilaroxazine's interaction potential with 11 clinically relevant drug transporters as a substrate and inhibitor. These included efflux transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and bile salt export pump (BSEP); and uptake transporters multi-drug and toxin extrusion 1 (MATE1), MATE2-K, organic anion transporter 1 (OAT1), OAT3, organic cation transporter 1 (OCT1), OCT2, organic anion transporting polypeptide 1B1 (OATP1B1), and OATP1B3.

## METHODS

*Transporter Expressing Cell Lines*

P-gp and BCRP substrate/inhibitor studies involved Madin-Darby canine kidney (MDCKII) epithelial cells (Sigma-Aldrich, St. Louis, MO). OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K evaluation involved human embryonic kidney (HEK293) cells expressing these transporters, BSEP evaluation involved HEK293-derived human BSEP membrane vesicles, and mock HEK293 cells as controls (Corning Life Sciences, Bedford, MA).

*Transporter Control Substrates and Inhibitors*

The study employed standard inhibitors and substrates as controls (Table 1).

**Table 1. Positive Substrates and Positive Control Inhibitors of Transporters**

| Transporter | Substrate | Inhibitor |
|---|---|---|
| P-gp | Digoxin (5 µM) | Ketoconazole (50 µM) |
| BCRP | Prazosin (5 µM) | Ko-143 (10 µM) |
| BSEP | TCA (10 µM) | Ketoconazole (100 µM) |
| OATP1B1 | E17G (2 µM) | Rifampin (100 µM) |
| OATP1B3 | E17G (5 µM) | Rifampin (100 µM) |
| OAT1 | PAH (5 µM) | Diclofenac (200 µM) |
| OAT3 | E3S (5 µM) | Probenecid (100 µM) |
| OCT1 | Metformin (100 µM) | Verapamil (50 µM) |
| OCT2 | Metformin (100 µM) | Amitriptyline (50 µM) |
| MATE1 | Metformin (100 µM) | Cimetidine (100 µM) |
| MATE2-K | Metformin (100 µM) | Pyrimethamine (10 µM) |

Brilaroxazine concentrations tested in the substrate assays were 3, 10, and 30 µM, and in the inhibition assays ranged from 0-100 µM.

*Substrate and Inhibition Assays*

The investigation performed all substrate and inhibition assays using established procedures. Concentration determination of brilaroxazine and control substrates involved using LC-MS/MS analysis (Sciex API 4000 and 4000 QTrap systems).

*Data Analysis*

The analysis utilized the following equations to calculate the Apparent Permeability ($P_{app}$) and Efflux Ratio (ER):

- $P_{app} = (dC_R/dt \times V_R)/(S \times C_{D,\,0hr})$
- ER = $P_{app}$ (B to A)/$P_{app}$(A to B)

Where $dC_R/dt$ = rate of appearance of substrate or test article in the receiver chamber; S = surface area of the cell membrane; $C_{D,\,0hr}$ = initial drug concentration in the donor chamber; $P_{app}$ (B to A) = apparent permeability from basal to apical membrane; $P_{app}$ (A to B) = apparent permeability from apical to basal membrane.

Analysis calculated the $IC_{50}$ using non-linear curve fittings with four parameters logistic equation:

$$y = d + \frac{a - d}{\left[(1 + (\frac{x}{c})^b\right]}$$

## RESULTS

### Table 2

**Table 2. Apparent Permeability (Papp) and Efflux Ratio (ER) of Brilaroxazine in P-gp and BCRP Expressed MDCKII Cell Monolayers**

| Transporter | Substrate | Concentration (mM) | (-) Inhibitor[1] | | | (+) Inhibitor[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | | $P_{app}$ ($10^{-6}$ cm/sec) | | ER | $P_{app}$ ($10^{-6}$ cm/sec) | | ER |
| | | | A to B | B to A | | A to B | B to A | |
| P-gp | Brilaroxazine | 3 | 0.24 | 2.16 | 9.18 | 2.71 | 5.12 | 1.89 |
| | | 10 | 0.99 | 3.51 | 3.54 | 3.18 | 5.37 | 1.69 |
| | | 30 | 2.76 | 4.72 | 1.71 | 4.55 | 6.39 | 1.40 |
| | Digoxin | 5 | 0.65 | 6.37 | 9.73 | 1.83 | 2.18 | 1.20 |
| BCRP | Brilaroxazine | 3 | 0.54 | 2.34 | 4.33 | 1.03 | 2.57 | 2.50 |
| | | 10 | 1.46 | 2.22 | 1.52 | 2.20 | 4.10 | 1.86 |
| | | 30 | 3.03 | 4.30 | 1.42 | 3.51 | 5.91 | 1.68 |
| | Prazosin | 5 | 0.82 | 15.89 | 19.40 | 9.09 | 10.35 | 1.14 |

[1] The investigation utilized ketoconazole as the P-gp inhibitor and Ko 143 as the BCRP inhibitor.

In P-gp-expressed MDCKII cells, the ERs of brilaroxazine were 9.18, 3.54 and 1.71 at 3, 10 and 30 µM, respectively. The ER decreased by >50% in the presence of a P-gp inhibitor, ketoconazole, indicating that brilaroxazine is a substrate of P-gp. Similarly, in BCRP-expressed MDCKII cells, the ERs of brilaroxazine were 4.33, 1.52 and 1.42 at 3, 10 and 30 µM, respectively, suggesting that brilaroxazine is a substrate of BCRP.

Evaluations with all other transporters, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K and BSEP, failed to demonstrate any significant evidence of substrate interaction with brilaroxazine.

### Table 3

**Table 3. Inhibition of Transporters by Brilaroxazine**

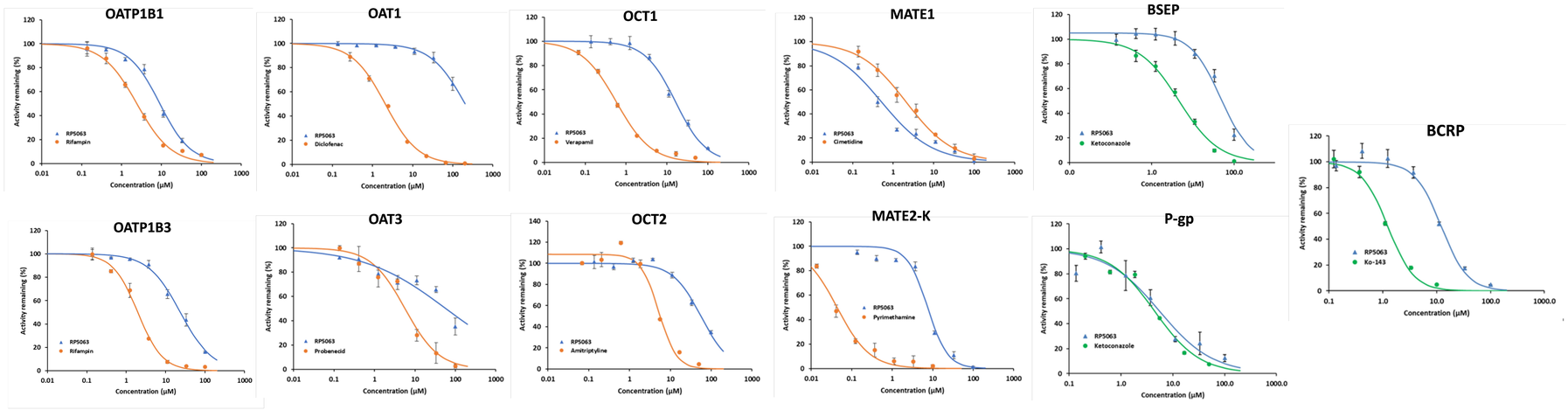
| Transporter | IC50 (µM) | Substrate |
|---|---|---|
| P-gp | 5.7 | Digoxin (5 µM) |
| BCRP | 12.3 | Prazosin (5 µM) |
| BSEP | 46.9 | TCA (10 µM*) |
| OATP1B1 | 9.1 | E17G (2 µM) |
| OATP1B3 | 23.6 | E17G (5 µM) |
| OAT1 | >100 | PAH (5 µM) |
| OAT3 | 58.4 | E3S (5 µM) |
| OCT1 | 16.3 | Metformin (100 µM) |
| OCT2 | 56.4 | Metformin (100 µM) |
| MATE1 | 0.5 | Metformin (100 µM) |
| MATE2-K | 7.5 | Metformin (100 µM) |

Brilaroxazine inhibited OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE2-K, BSEP, P-gp, and BCRP with $IC_{50}$ values ranging from 5.7 µM to >100 µM. MATE1 was the only transporter with an $IC_{50}$ of <1 µM.

*Concentration of cholic acid, converted to TCA *in situ*

### Figure 1

**Figure 1. Brilaroxazine as Inhibitor of Transporters**




Inhibition curves for OAT1B1, OAT1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, BSEP, P-gp and BCRP by brilaroxazine and prototypical inhibitors. All transporter inhibition studies utilized bilaroxazine concentrations of 0, 0.1, 0.4, 1.2, 3.7, 11.1, 33.3 and 100 µM. For control inhibitors, a concentration range of 0-10 µM was used for pyrimethamine (MATE2-K) and Ko-143 (BCRP), 0-50 µM for verapamil (OCT1), amitriptyline (OCT2), and ketoconazole (P-gp), 0-100 µM for rifampin (OATP1B1 and OATP1B3), probenecid (OAT3), cimetidine (MATE1), and ketoconazole (BSEP) and 0-200 µM for diclofenac (OAT1).

## DISCUSSION

*Significance of Transporter Inhibition by Brilaroxazine*

The scientific and regulatory community recognizes that drug transporters—solute carriers and ATP-binding cassette types—can affect drug PK, safety, and efficacy.[12-14] While drug-metabolizing enzyme activity occurs primarily in the liver and small intestines, transporter expression occurs in tissues throughout the whole human body.[13,14] Because they can govern the access of endogenous and exogenous substances to various sites in the body, it is essential to evaluate transporter-mediated interactions with new pharmaceutical compounds.[14]

Regulatory agencies have recommended that sponsors evaluate their investigational drugs as substrates and/or inhibitors of clinically relevant transporters. Accordingly, this work sought to address this requirement[12-14] and evaluated 11 clinically relevant transporters.

*In vitro*, brilaroxazine is a substrate of efflux transporters P-gp and BCRP. Co-administration with strong inhibitors/inducers of these transporters may increase/decrease its systemic exposure. In a clinical drug interaction study with itraconazole, a potent inhibitor of P-gp and BCRP, the study observed no altered PKs of brilaroxazine, indicating that the *in vitro* data did not translate *in vivo* in humans.[11]

Brilaroxazine is not a substrate of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1. One should not expect substrate interactions with MATE2-K and BSEP and drug interactions when brilaroxazine is co-administered with inhibitors or inducers of the above transporters.

Brilaroxazine inhibited P-gp and BCRP with $IC_{50}$ values of 5.7 and 12.3 µM. Based on FDA[15] and EMA[16] guidance documents, a DDI is possible due to inhibition of P-gp and BCRP at the gastrointestinal (GI) tract when $I_{gut}/IC_{50}$ is ≥10, where $I_{gut}$ is the luminal concentration of the interacting drug calculated as the dose/250 mL or, if low solubility, the maximum possible concentration at the pH range of the GI tract (EMA only) and $IC_{50}$ is the half-maximal inhibitory concentration. For P-gp and BCRP inhibition, the $I_{gut}/IC_{50}$ values were >10 based on the highest brilaroxazine therapeutic dose of 50 mg, suggesting that there may be a potential for DDI in the GI tract. However, based on the $I_1/IC_{50}$, where $I_1$ is the $C_{max,\,ss}$ at the highest therapeutic dose of 50 mg, and $IC_{50}$ is the half-maximal inhibitory concentration, systemic DDI is unlikely to occur.

Brilaroxazine inhibited the hepatic uptake transporters OATP1B1, OATPIB3 and OCT1 with $IC_{50}$ values of 9.1, 23.6 and 16.3 µM, respectively. The R values estimated based on the steady-state exposure at the highest therapeutic dose of 50 mg suggest that DDI is unlikely when brilaroxazine is co-administered with substrate drugs of OATP1B1, OATP1B3 and OCT1.[15] Brilaroxazine inhibited the hepatic efflux transporter BSEP with an $IC_{50}$ of 46.9 µM. Since the 50-fold unbound $C_{max,\,ss}$ (estimated liver concentration) is well below the $IC_{50}$ value, it is unlikely that brilaroxazine will inhibit BSEP clinically.[16]

Brilaroxazine inhibited the renal transporters OAT1, OAT3, OCT2, MATE1 and MATE2-K with $IC_{50}$ values of >100, 58.4, 56.4, 0.5 and 7.5 µM. Based on the $I_{max,u}/IC_{50}$ (<0.1) at the highest therapeutic dose of 50 mg, DDI is unlikely when brilaroxazine is co-administered with substrate drugs of the above transporters.[16]

## CONCLUSION

This study evaluated *in vitro* the interaction between brilaroxazine and 11 clinically relevant transporters. The results found that brilaroxazine displays limited interaction with clinically relevant drug transporters. Such findings indicate that in most cases, the potential for transporter-mediated DDI appears to be unlikely at the highest therapeutic dose. Further work in the clinic will help to confirm these *in vitro* findings.

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**Disclosures:**

Laxminarayan Bhat, PhD and Seema R Bhat, MSc are employees. Palaniappan Kulanthaivel, PhD is a consultant for Reviva Pharmaceutical Holdings, Inc.

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