Multiple Computational Approaches for Predicting Drug Interactions with Human Equilibrative Nucleoside Transporter 1

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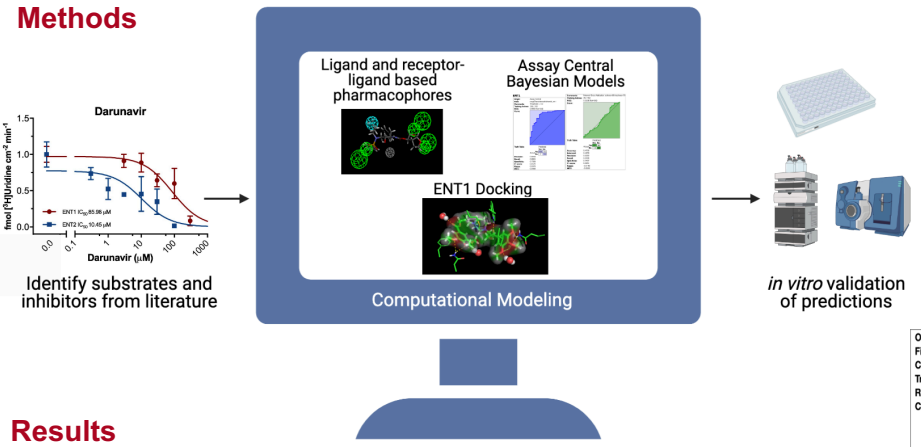
Abstract

ENTs participate in the pharmacokinetics and disposition of nucleoside analog drugs to sanctuary sites, including the male genital tract. Understanding drug-interactions with the ENTs may inform and facilitate the development of new drugs, including chemotherapeutics and antivirals that require access to the male genital tract. This study created 3-D pharmacophores for ENT1 and ENT2 substrates and inhibitors, using K_t and IC_{50} data curated from the literature. Substrate pharmacophores for ENT1 and ENT2 are distinct, with partial overlap of hydrogen bond donors, and small compared with the inhibitor pharmacophores (that predominantly feature hydrogen bond acceptors). Mizoribine mapped to the ENT1 substrate pharmacophore and proved to be a substrate of the ENTs; the presence of the ENT-specific inhibitor, NBMPR, decreased mizoribine accumulation in ENT1 and ENT2 cells (ENT1, ~70% decrease, 38.7 pmol cm^{-2} vs 11.4 pmol cm^{-2}; p = 0.0046; ENT2, ~50% decrease 21.09 pmol cm^{-2} vs. 10.7 pmol cm^{-2}; p = 0.0012). Darunavir mapped to the ENT1 inhibitor pharmacophore and its interaction with ENTs was limited to inhibition; NBMPR did not significantly influence darunavir accumulation in either ENT1 or ENT2 cells (ENT1, 74.6 vs. 66.9 pmol cm^{-2}, p = 0.28; ENT2, 65.3 vs. 61.1 pmol cm^{-2}, p = 0.53), indicating that darunavir's interaction with the ENTs is restricted to inhibition. These models can inform compound selection in the drug discovery and development process, thereby reducing time and expense of identification and optimization of ENT interacting compounds that predict antiviral and chemotherapeutic penetration into the MGT.

Objective

Generate multiple computational methods for predicting interactions with the ENTs at the blood-testis barrier and validate these models with *in vitro* transport experiments.

Methods



Results

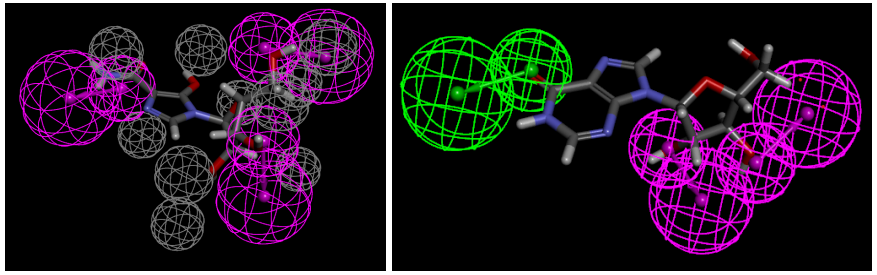


Figure 1: Substrate Pharmacophore Mapping. Mizoribine (left) mapped to ENT1 substrate pharmacophore and inosine (right) mapped to ENT2 substrate pharmacophore. Purple = hydrogen bond donors, green = hydrogen bond acceptors, grey = excluded volumes. **Mizoribine is predicted to be an ENT1 substrate. Inosine is predicted to be an ENT2 substrate.**

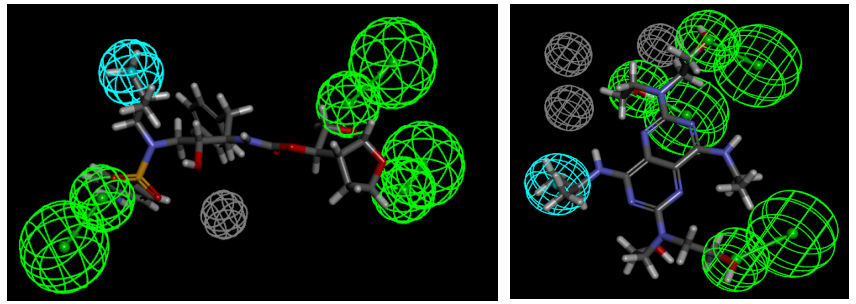
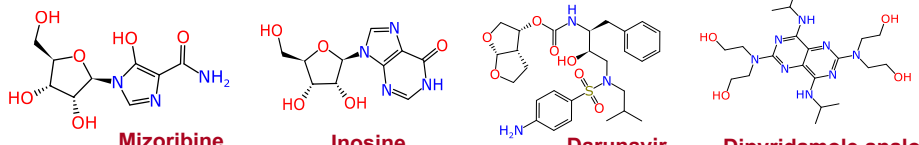


Figure 2: Inhibitor Pharmacophore Mapping. Darunavir (left) mapped to the ENT1 inhibitor pharmacophore and a dipyridamole analog (right) mapped to the ENT2 inhibitor pharmacophore. Cyan features = hydrogen bond donors, green features = hydrogen bond acceptors, and grey features = excluded volumes. **Darunavir is predicted to be an ENT1 inhibitor. This dipyridamole analog is predicted to be an ENT2 inhibitor.**

Pharmacophore	Compound	Fit Value	Estimate
ENT1 Substrate	Mizoribine	5.7	K_t 12.3 μM
ENT1 Inhibitor	Darunavir	4.5	IC_{50} 30.8 μM

Table 1: Fit values and estimated parameters for mizoribine and darunavir.



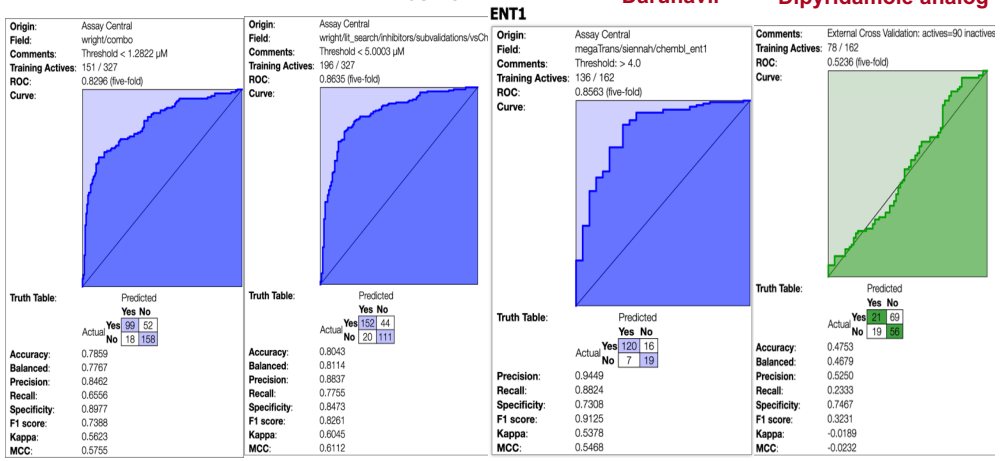


Figure 3: **Bayesian Modeling.** A) Bayesian model integrating ChEMBL data with literature data. B) ENT1 ChEMBL training set in purple. Calculated threshold of 1.9 μM to analyze literature inhibitor data in green. **Bayesian models were created to predict ENT interactions.**

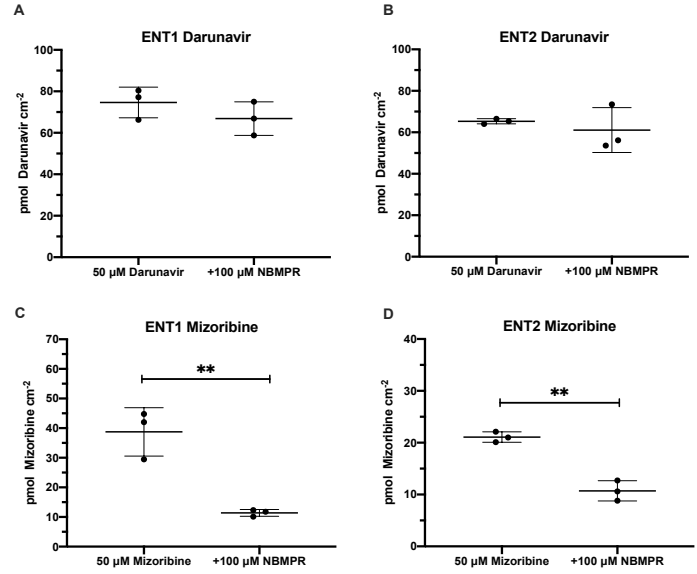


Figure 4: Validation of Predictions. Darunavir uptake in ENT1 (A) and ENT2 (B) cells. Mizoribine uptake in ENT1 (C) and ENT2 (D) cells. **Mizoribine is an ENT substrate. Darunavir is an ENT inhibitor.**

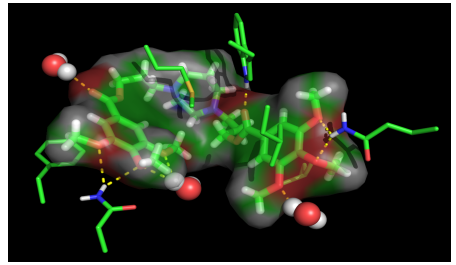


Figure 5: ENT1 Docking. NBMPR docked to ENT1 crystal structure.

Conclusions

- Mizoribine was a predicted and confirmed as an ENT1 substrate.
- Darunavir was predicted and confirmed as an ENT1 inhibitor.
- These computational models accurately predict interactions with the ENTs at the BTB.

Funding

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References

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