Validating and optimizing *in vitro* assays for improved DDI prediction – assay calibration and time-dependent inhibition

MEEX, Cambridge, MA / Sep 5, 2019

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Solvo Biotechnology

The Magic Formula of Three Unknowns

$[I_{1T}] = I_{max}$

$[I_{1u}] = I_{max} \times f_{u,p}$

$[I_2] = \text{Dose} / 250\ \text{mL}$

$[I_{in,max}] = I_{max} + (F_a F_g \times k_a \times \text{Dose})/Q_h/R_B$

$$\frac{[I]}{IC_{50}} \geq \tau$$

- ML
  - ER
  - P_{app}
- VT
- OE cell line
- hepatocyte

Substrate?

	FDA	EMA
OATP1B1/3	$I_{inlet,max,u}/IC_{50} \geq 0.1$	$I_{inlet,max,u}/IC_{50} \geq 0.04$
OAT1/3, OCT2	$I_{max,u}/IC_{50} \geq 0.1$	$I_{max,u}/IC_{50} \geq 0.02$
MATE1/2-K	$I_{max,u}/IC_{50} \geq 0.02$	$I_{max,u}/IC_{50} \geq 0.02$
OCT1	-	$I_{inlet,max,u}/IC_{50} \geq 0.04$

"Sponsors may calibrate their internal *in vitro* systems with known inhibitors and non-inhibitors of these transporter systems and propose a specific cutoff value with proper justification." (FDA Draft Guidance)

Art → Craft → Science

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Topic #1

Optimized workflow for the identification of MDR1 inhibitors

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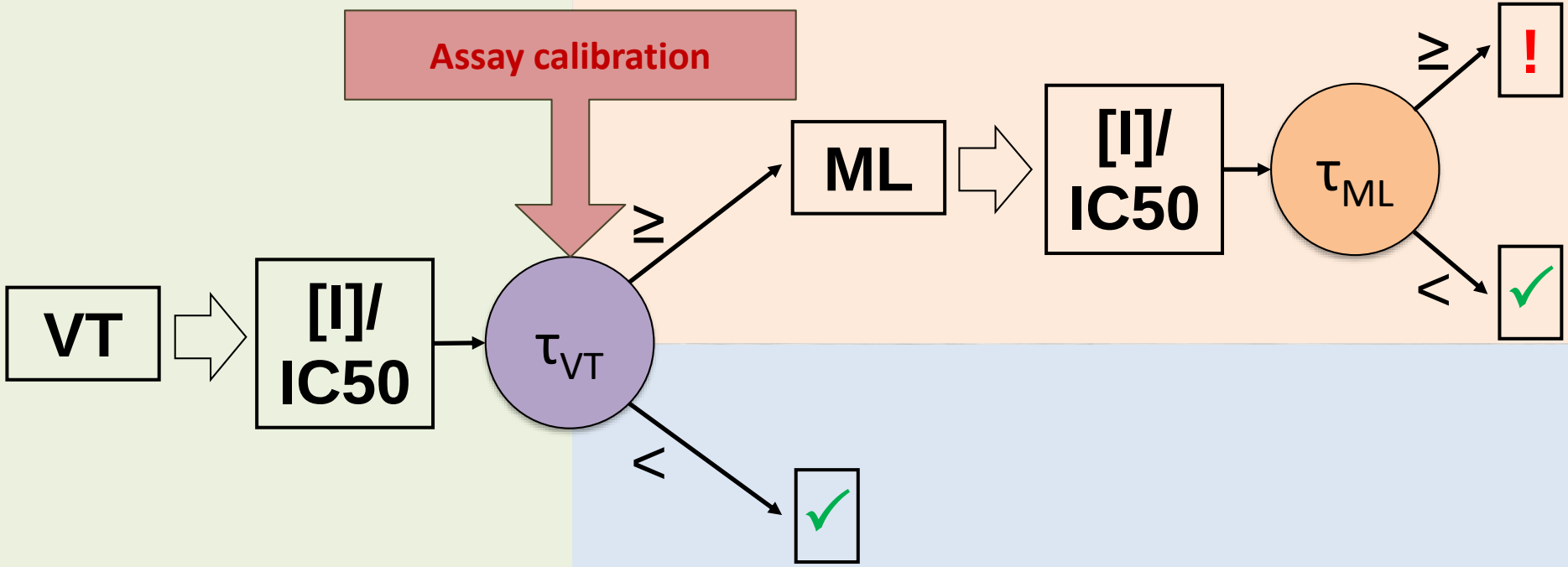
ML vs. VT for determining MDR1 IC_{50}

Monolayer	Vesicular transport
Need for cell culturing: extended timeline, sensitive to conditions	Vesicles mass producible & stockable: ready-to-use assay
Physiologically more relevant: tight cellular barrier	Acellular system, physiologically less relevant
Pharmacological substrates: digoxin, fexofenadine, talinolol	Non-pharmacological substrate: NMQ
Inhibitor permeation may be limited: risk of false negative prediction for low passive perm cpds	IC aspect of transporter freely accessible: accepts any cpd Risk of false positives
High costs	Cheaper

A two-tier decision scheme



Calibration of the MDR1 inhibition assay

- Roche (Poirier et al., DMD 2014)
  - 57 molecules, of which 33 clinically relevant digoxin interactors (AUC_i/AUC or $C_{max,i,ss}/C_{max,ss} > 1.25$)
  - Model system: LLC-PK1-MDR1 ML; substrate: digoxin

- SOLVO
  - 32 molecules – a subset of the Roche library
  - 1st tier: HEK293-MDR1 VT; substrate: N-methylquinidine
  - TARGET: achieve the same sensitivity as the Roche ML system
  - 2nd tier: cAbcb1^{KO}-MDCKII-MDR1 ML (work in progress – initial results)

Calibration of the MDR1 VT inhibition assay

			Roche LLC-PK1-MDR1 ML / digoxin			SOLVO HEK293-MDR1 VT / ^{3}H-N-methylquinidine		
	AUC_i/AUC	$C_{max,i,ss}/C_{max,ss}$	$[I_{1T}]/IC_{50}$	$[I_{1u}]/IC_{50}$	$[I_2]/IC_{50}$	$[I_{1T}]/IC_{50}$	$[I_{1u}]/IC_{50}$	$[I_2]/IC_{50}$
Cutoff	**1.25**	**1.25**	**0.1**	**0.02**	**10**	**0.1**	**0.02**	**10**
Aliskiren	0.86	0.92	0.00145	0.00073	7.25	0.28126	0.14204	1406.40
Carvedilol	1.24	1	0.10000	0.00500	47.31	0.03879	0.00194	18.35
Erythromycin	0.96	1	0.01567	0.00235	3.63	0.07786	0.01168	18.06
Meloxicam	1.03	1.03	0.00271	0.00003	0.09	0.02705	0.00027	0.85
Rosuvastatin	1.04	1.04	0.00006	0.00001	0.53	0.00001	0.00000	0.10
Sertraline	1.1	1.05	0.03023	0.00030	202.51	0.00582	0.00006	38.99
Troglitazone	1.04	1.05	0.22767	0.00228	227.90	0.25368	0.00254	253.94
Nifedipine	1.23	1.06	0.00398	0.00016	2.00	0.00855	0.00034	4.29
Omeprazole	1.1	1.1	0.02000	0.00100	2.11	0.00677	0.00034	0.71
Pantoprazole	1.11	1.1	0.01317	0.00026	0.84	0.01164	0.00023	0.75
Losartan	1.06	1.13	0.00163	0.00002	1.58	0.00148	0.00002	1.43
Atorvastatin	1.15	1.2	0.00041	0.00001	0.72	0.02139	0.00043	37.12
Mibefradil	1.08	1.22	0.27018	0.00270	212.40	0.06859	0.00069	53.92
Nitrendipine	1.09	1.22	0.00082	0.00001	6.10	0.00045	0.00000	3.30
Dipyridamole	1.08	1.23	0.17209	0.00172	55.30	0.69133	0.00691	222.16
Tolvaptan	1.178	1.274	0.03707	0.00074	20.64	0.11123	0.00222	61.94
Etoricoxib	1.06	1.33	0.07692	0.00615	10.29	0.03126	0.00250	4.18
Propafenone	-	1.33	0.03202	0.00320	86.55	0.02502	0.00250	67.63
Felodipine	1.18	1.34	0.00714	0.00007	24.76	0.00131	0.00001	4.54
Itraconazole	1.68	1.34	0.52778	0.00528	630.00	5.21405	0.05214	6223.93
Diltiazem	1.44	1.38	0.15217	0.03348	125.86	0.01491	0.00328	12.33
Cyclosporin A	-	1.44	2.54545	0.11455	1060.91	16.74641	0.75359	6979.67
Verapamil	1.51	1.44	0.42857	0.03986	251.39	0.38053	0.03539	223.21
Talinolol	1.23	1.45	0.00103	0.00051	1.17	0.00851	0.00417	9.67
Telmisartan	1.22	1.58	0.09098	0.00091	76.45	1.42080	0.01421	1193.86
Amiodarone	1.63	1.72	0.25938	0.00259	258.27	0.41972	0.00420	417.94
Quinidine	1.76	1.75	0.72245	0.11559	693.24	0.69453	0.11112	666.45
Conivaptan	1.43	1.79	0.06988	0.00070	19.34	4.68308	0.04683	1295.92
Clarithromycin	1.64	1.83	0.06801	0.03401	33.68	0.17834	0.08917	88.31
Valspodar	3.05	2.44	4.05263	3.44474	1733.25	15.74159	13.38035	6732.46
Darunavir	1.77	-	0.03065	0.00153	13.47	0.62729	0.03136	275.73
Dronedarone	1.41	-	0.27429	0.00274	4105.36	0.28120	0.00281	4208.77

Accuracy	**0.59**	**0.66**	**0.81**	**0.75**	**0.69**	**0.66**
Sensitivity	**0.47**	**0.35**	**0.94**	**0.71**	**0.47**	**0.82**
Specificity	**0.73**	**1.00**	**0.67**	**0.80**	**0.93**	**0.47**

Calibration of the MDR1 VT inhibition assay

			Roche LLC-PK1-MDR1 ML / digoxin			SOLVO HEK293-MDR1 VT / ^{3}H-N-methylquinidine		
	AUC_i/AUC	$C_{max,i,ss}/C_{max,ss}$	$[I_{1T}]/IC_{50}$	$[I_{1u}]/IC_{50}$	$[I_2]/IC_{50}$	$[I_{1T}]/IC_{50}$	$[I_{1u}]/IC_{50}$	$[I_2]/IC_{50}$
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Aliskiren	0.86	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Carvedilol	1.24							
Erythromycin	0.96							
Meloxicam	1.03							
Rosuvastatin	1.04							
Sertraline	1.1							
Troglitazone	1.04							
Nifedipine	1.23							
Omeprazole	1.1							
Pantoprazole	1.11							
Losartan	1.06							
Atorvastatin	1.15							
Mibefradil	1.08							
Nitrendipine	1.09							
Dipyridamole	1.08							
Tolvaptan	1.178							
Etoricoxib	1.06							
Propafenone	-							
Felodipine	1.18							
Itraconazole	1.68							
Diltiazem	1.44							
Cyclosporin A	-							
Verapamil	1.51							
Talinolol	1.23							
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Amiodarone	1.63							
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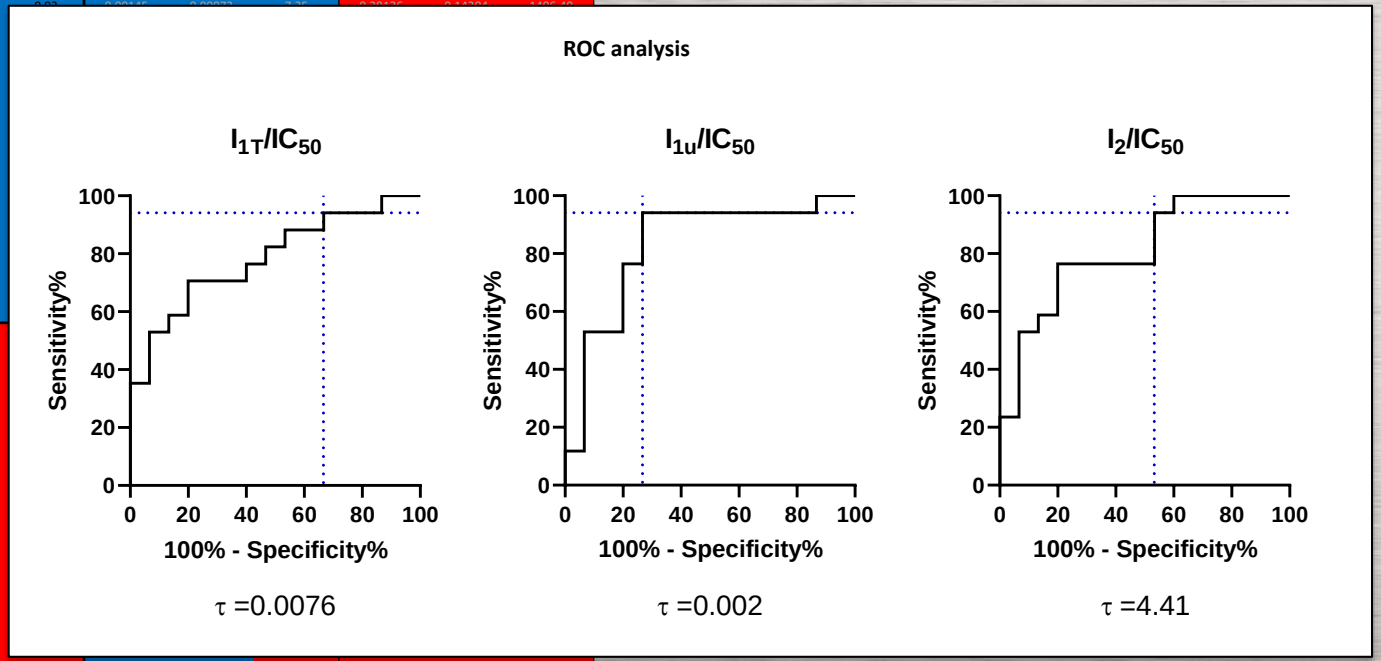

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Erythromycin	0.96	1	0.01567	0.00235	3.63	0.07786	[illegible]	[illegible]
Meloxicam	1.03	1.03	0.00271	0.00003	0.09	0.02705	0.00027	0.85
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Sensitivity	0.47	0.35	0.94	0.71	0.47	0.82
Specificity	0.73	1.00	0.67	0.80	0.93	0.47

Clinically relevant inhibitor in select populations

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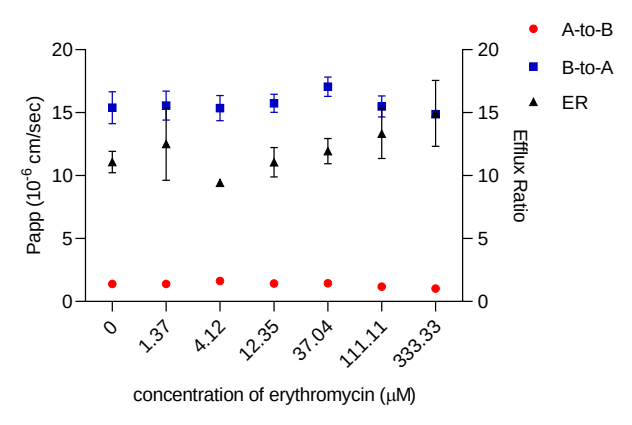
Dronedarone	1.41	-	0.27429	0.00274	4105.36	0.28120	0.00281	4208.77

Accuracy	0.66	0.66	0.78	0.66	0.84	0.72
Sensitivity	0.94	0.94	0.94	0.94	0.94	0.94
Specificity	0.33	0.33	0.60	0.33	0.73	0.47

MDCKII validation: the system and first results

		Digoxin ER, mean ± SD	OE / Ctrl
Former	Ctrl: MDCKII	4.44 ± 0.58	4.54
	OE: MDCKII-MDR1	20.15 ± 1.03	
Current	Ctrl: cAbcb1^{KO}-MDCKII-Mock	0.84 ± 0.01	22.3
	OE: cAbcb1^{KO}-MDCKII-MDR1	18.75 ± 2.01	

Erythromycin / Digoxin



IC_{50}(ER) > 333 μM

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Conclusions of Topic #1

- A comparatively fast and inexpensive VT assay of MDR1 inhibition, when appropriately calibrated, could achieve the same sensitivity as ML assays
- NMQ, albeit a non-pharmacological substrate, did not lack the adequate sensitivity for detecting clinically relevant MDR1 inhibition
- Using VT as a first screen and ML for confirming or refuting positives may be a viable strategy

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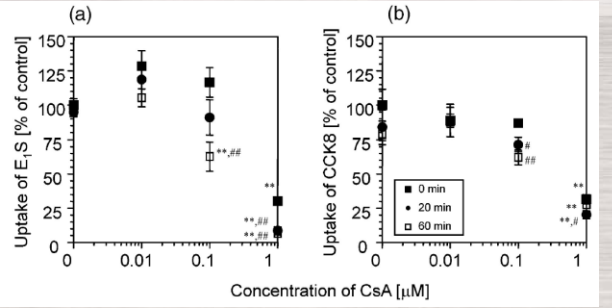
Topic #2

Considering time-dependent inhibition (Potentiation of Transporter Inhibition by Preincubation) to avoid false negative DDI predictions

Tatrai et al. *Drug Metab Dispos.* 2019 Jul;47(7):768-778.

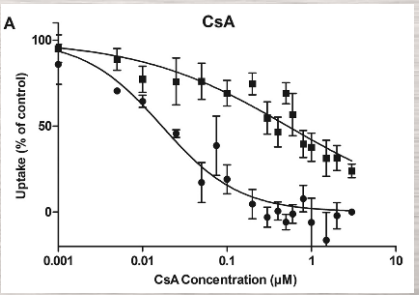
PTIP – Background

- The inhibitory effect of cyclosporin A (CsA) on OATP1B1 and 1B3 was enhanced by pretreatment



Shitara et al. *Drug Metab Pharmacokinet.* 2012;27(4):368-78.

- For OATP1B1-mediated atorvastatin transport, a 1-hour preincubation with CsA resulted in a 20-fold reduction of IC_{50}



Amundsen et al. *Drug Metab Dispos.* 2010 Sep;38(9):1499-504.

→ FDA recommends a minimum of 30-min preincubation to determine IC_{50} on OATP1B1/1B3

Does PTIP affect OATP1Bs only?

Inhibitor	OATP1B1 1 µM E2-17βDG, 3 min			OATP1B3 1 µM E2-17βDG, 3 min		
	IC_{50} (µM)		PTIP	IC_{50} (µM)		PTIP
	w/o pre	w/ pre		w/o pre	w/ pre	
Venetoclax	>171	0.663	**>258**	>300	34.5	**>8.70**
Cyclosporin A	0.667	0.0983	**6.78**	0.505	0.167	**3.02**
Saquinavir	3.65	1.03	**3.54**	11	2.91	**3.78**
Atorvastatin	1.68	0.892	**1.88**	0.71	0.314	**2.26**
Rifampicin	1.2	0.818	**1.47**	3.67	2.13	**1.72**
Gemfibrozil	32.9	33.7	**0.976**	246	366	**0.672**

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Inhibitor	OAT1 5 µM pAH, 2 min			OAT3 1 µM E3S, 1 min		
	IC_{50} (µM)		PTIP	IC_{50} (µM)		PTIP
	w/o pre	w/ pre		w/o pre	w/ pre	
Benzbromarone	2.68	1.47	**1.82**	5.76	3.82	**1.51**
Furosemide	45.5	31.7	**1.44**	5.98	8.28	**0.723**
Valsartan	22.7	16.2	**1.4**	3.57	2.26	**1.58**
Probenecid	14.4	10.5	**1.37**	3.01	1.91	**1.58**
Diclofenac	8.64	6.89	**1.25**	3.1	7.24	**0.428**
Bumetanide	50.8	42.6	**1.19**	3.35	2.53	**1.32**
Gemfibrozil	12.7	24.7	**0.514**	7.46	6.14	**1.21**
Rifampicin	>100	>100	N/D	>100	>100	N/D
Saquinavir	>100	>100	N/D	>100	>100	N/D
Pravastatin	>300	>300	N/D	>300	>300	N/D

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Does PTIP affect OATP1Bs only?

Inhibitor	OCT1 10 µM metformin, 5 min IC$_{50}$ (µM) w/o pre	OCT1 IC$_{50}$ (µM) w/ pre	OCT1 PTIP	OCT2 10 µM metformin, 1 min IC$_{50}$ (µM) w/o pre	OCT2 IC$_{50}$ (µM) w/ pre	OCT2 PTIP
Ledipasvir	>50	0.196	**>255**	>200	22.9	**>8.73**
Irinotecan	0.121	0.0223	**5.43**	57.3	27	**2.12**
Saquinavir	18.2	4.45	**4.09**	>100	>100	N/D
Daclatasvir	0.533	0.0943	**5.65**	67.8	1.98	**34.2**
Verapamil	0.459	0.15	**3.06**	36.2	19.5	**1.86**
Isavuconazole	5.21	1.75	**2.98**	16.8	1.27	**13.2**
Vandetanib	5.22	1.94	**2.69**	19.8	4.72	**4.19**
Cetirizine	91.5	51.3	**1.78**	113	73.1	**1.55**
Cimetidine	150	87.6	**1.71**	>300	>300	N/D
Amisulpride	25.3	15.7	**1.61**	150	127	**1.18**
Ranolazine	66.9	49.5	**1.35**	235	218	**1.08**
Trimethoprim	27.8	20.6	**1.35**	98.3	77	**1.28**
Abacavir	99.9	112	**0.892**	>300	>300	N/D
Dolutegravir	>300	>300	N/D	15.9	1.41	**11.3**

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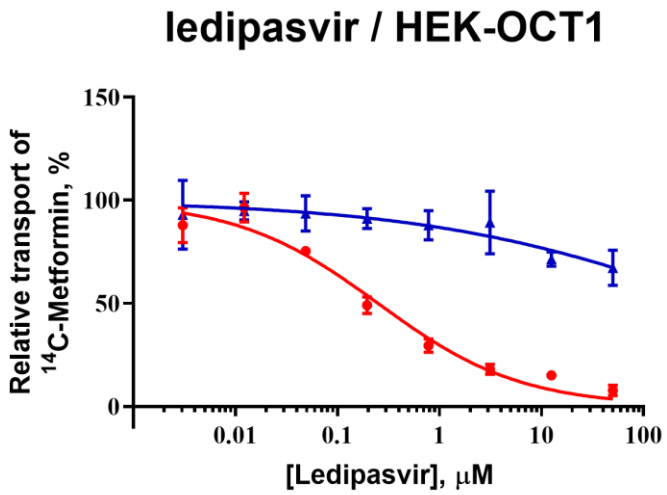
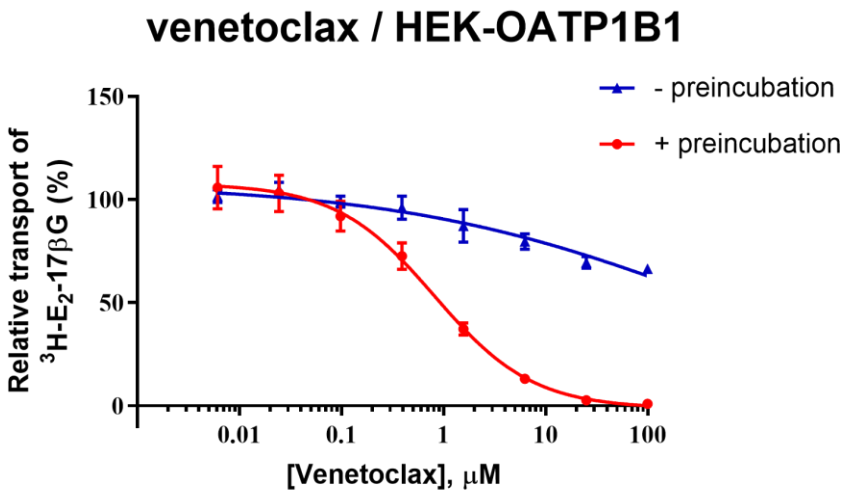
Inhibitor	MATE1 10 µM metformin, 15 min IC$_{50}$ (µM) w/o pre	MATE1 IC$_{50}$ (µM) w/ pre	MATE1 PTIP	MATE2-K 10 µM metformin, 15 min IC$_{50}$ (µM) w/o pre	MATE2-K IC$_{50}$ (µM) w/ pre	MATE2-K PTIP
Pyrimethamine	0.03614	0.01927	1.88	0.106	0.0507	2.09
Vandetanib	0.794	0.438	1.81	0.88	0.293	3.00
Trimethoprim	14.1	8.05	1.75	1.13	1.06	1.07
Ondansetron	0.0913	0.058	1.57	0.354	0.342	1.04
Isavuconazole	7.16	4.67	1.53	20.8	15.1	1.38
Cimetidine	4.32	3.41	1.27	7.54	10.1	0.747
Famotidine	1.63	1.45	1.12	54.1	31.2	1.73
Ranitidine	13.1	19.6	0.668	18.8	42.9	0.438

1 / 8

Most outstanding examples of PTIP



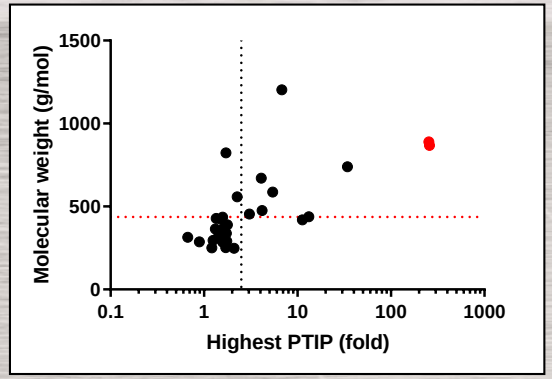


Is PTIP relevant to DDI predictions?

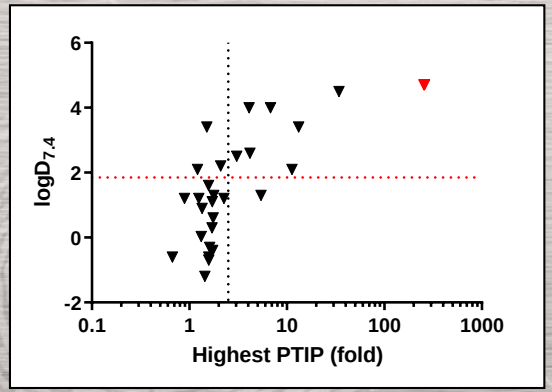
Perpetrator	Transporter	Victim	AUC_i / AUC	$C_{max,i}$ / C_{max}	[I]/IC50		FDA prediction			EMA prediction	
					w/o pre	w/ pre	w/o pre	w/ pre		w/o pre	w/ pre
Saquinavir (+ Ritonavir)	OATP1B1	Simvastatin	31.6	N/D	0.4583	1.6242					
Cyclosporin A	OATP1B1	Rosuvastatin	7.1	11	1.9938	13.5284					
Verapamil	OCT1	Metformin → Glucose	3.38	1.62	6.3806	19.5246					
Ledipasvir (+ Sofosbuvir)	OCT2	Tenofovir	1.98	1.79	0.0000	0.0002					
Dolutegravir	OCT2	Metformin	1.79	1.66	0.0068	0.0772					
Vandetanib	OCT2	Metformin	1.74	1.5	0.0101	0.0422					
Isavuconazole	OCT2	Metformin	1.52	1.23	0.0102	0.1346					
Daclatasvir	OCT2	Metformin	1.09	1.08	0.0003	0.0118					
Irinotecan	OCT2	N/D			0.0140	0.0296					?
Venetoclax	OATP1B1	N/D			0.0020	0.5156					

- Some clinical interactions are missed without preincubation → specific cutoff values should be calibrated with preincubation
- Positives with preincubation only: a trigger for clinical studies?

What molecular properties influence PTIP?



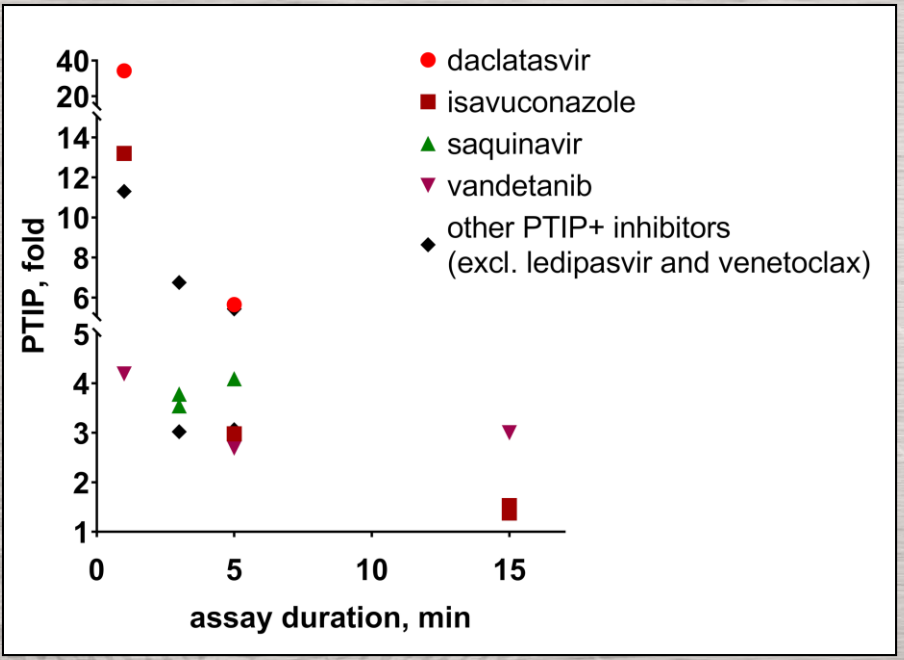
MW > 437 g/mol: PPV = 82%



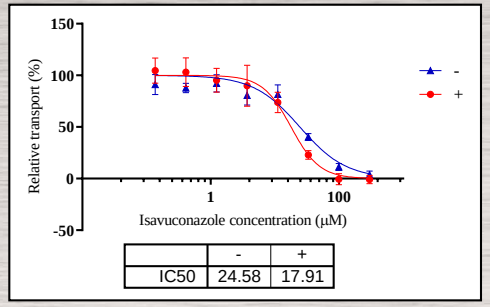
$LogD_{7.4}$ > 1.85: PPV = 75%

Inhibitor	Highest observed PTIP (fold)	Molecular weight (g/mol)	$LogD_{7.4}$	tPSA (Å)	cLogP	MDCK-LE P_{app} (10^{-6} cm/s)
Venetoclax	>258	868.5	4.7	174.7	10.0	0.15
Ledipasvir	>255	889	4.7	175	6.7	0.012
Daclatasvir	34.2	738.9	4.5	174.6	4.7	3.72
Isavuconazole	13.2	437.5	3.4	87.6	2.7	6.17
Dolutegravir	11.3	419.4	2.1	101	-0.4	9.51
Cyclosporin A	6.78	1202.6	4.0	279	14.0	3.71
Irinotecan	5.43	586.7	1.3	116.7	2.7	1.9
Vandetanib	4.19	475.4	2.6	59.5	3.7	8.45
Saquinavir	4.09	670.9	4.0	167	4.7	2.5
Verapamil	3.06	454.6	2.5	64	1.5	14.5
Atorvastatin	2.26	558.7	1.2	114	4.4	3.73
Pyrimethamine	2.09	248.7	2.2	77.8	2.4	11.7
Cetirizine	1.8	388.9	1.3	53	2.1	4.64
Trimethoprim	1.75	290.3	0.6	106	0.98	10.3
Famotidine	1.73	337.5	-0.4	176	-0.6	0.87
Rifampicin	1.72	823	1.1	220	1.5	1.44
Cimetidine	1.71	252.3	0.3	88.9	0.3	1.5
Amisulpride	1.61	369.5	-0.3	102	1.3	1.6
Probenecid	1.58	285.4	-0.7	74.7	3.4	7.31
Valsartan	1.58	435.5	-0.6	112	4.9	0.66
Ondansetron	1.57	293.4	1.6	44.8	2.1	16.9
Benzbromarone	1.51	424.1	3.4	50.4	6	4.2
Furosemide	1.44	330.7	-1.2	123	1.9	1.1
Ranolazine	1.35	427.5	0.9	74.3	1.0	16.8
Bumetanide	1.32	364.4	0.03	119	3.4	1.13
Diclofenac	1.25	296.2	1.2	49.3	4.7	16.7
Gemfibrozil	1.21	250.3	2.1	46.5	3.9	12.3
Abacavir	0.892	286.3	1.2	104	1.2	12.6
Ranitidine	0.668	314.4	-0.6	86.3	0.7	1.3
Pravastatin	N/A	424.5	-0.75	124	2.0	0.56

What assay conditions influence PTIP?

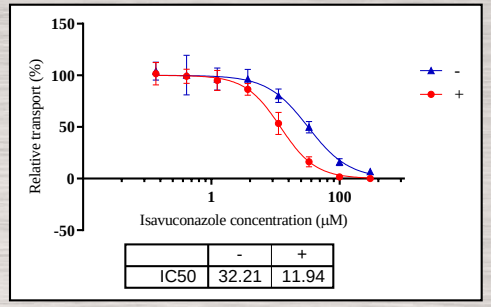


MDCKII-MATE2-K / Isavuconazole



	-	+
IC50	24.58	17.91

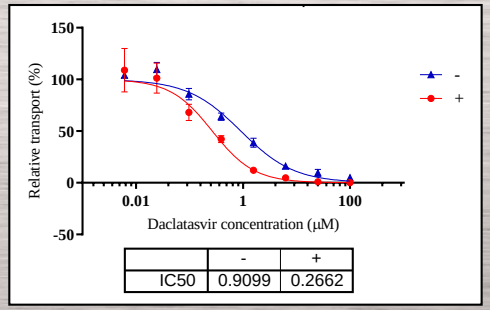
15-min assay: PTIP = **1.37**



	-	+
IC50	32.21	11.94

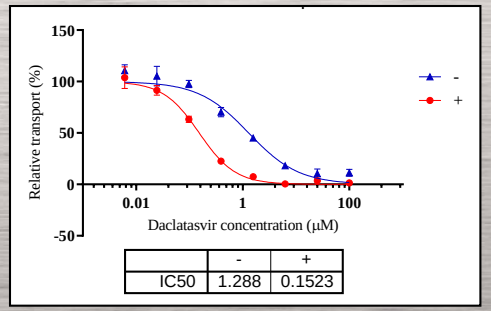
5-min assay: PTIP = **2.70**

HEK293-OCT2 / Daclatasvir



	-	+
IC50	0.9099	0.2662

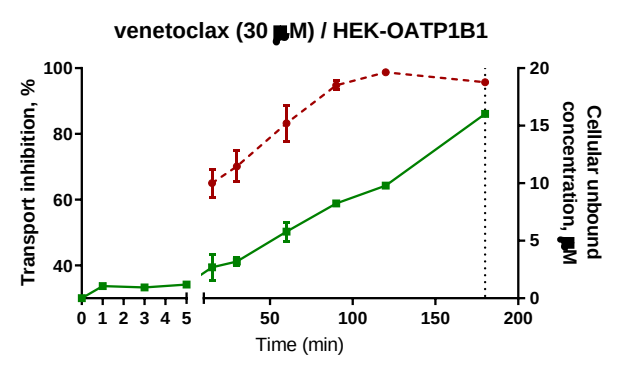
3-min assay: PTIP = **3.42**

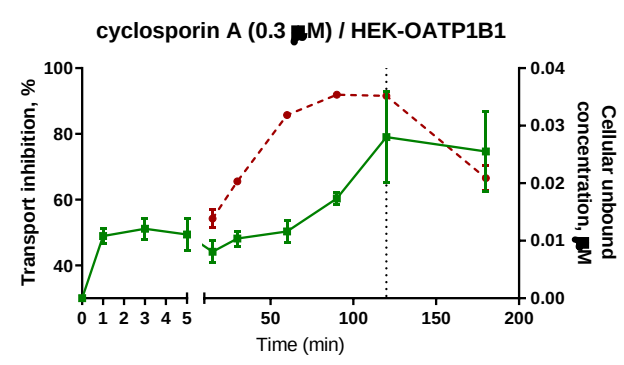


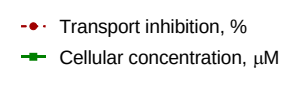
	-	+
IC50	1.288	0.1523

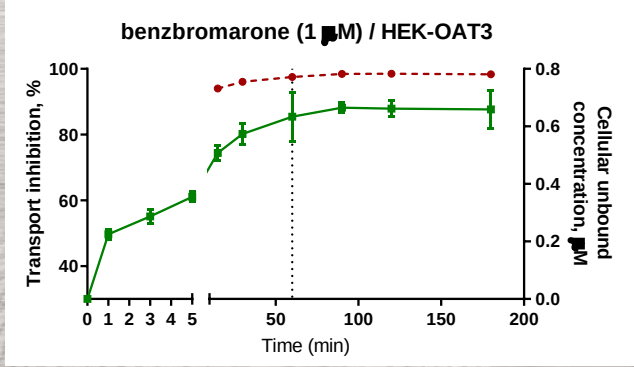
1-min assay: PTIP = **8.46**

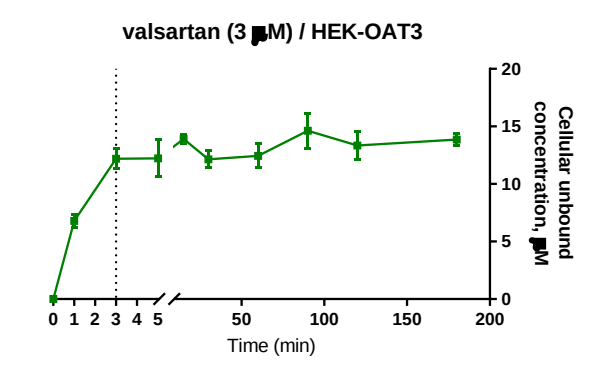
Is a 30-minute preincubation always enough?







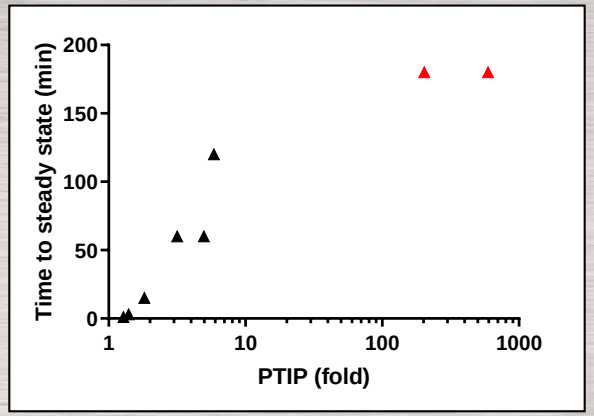


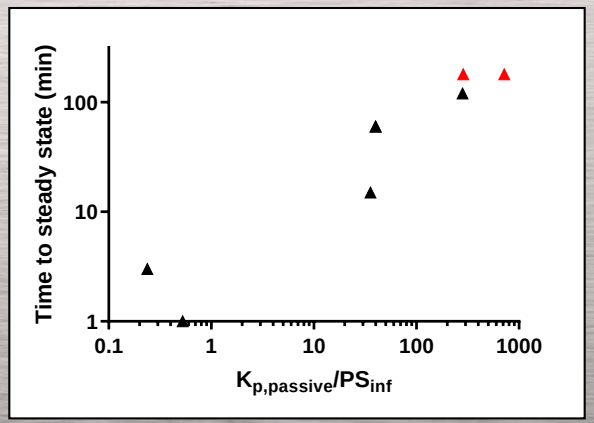


- Some drugs require >180 min to attain maximal inhibition
- Free inhibitor concentration in the cell, rather than in the medium, determines potency

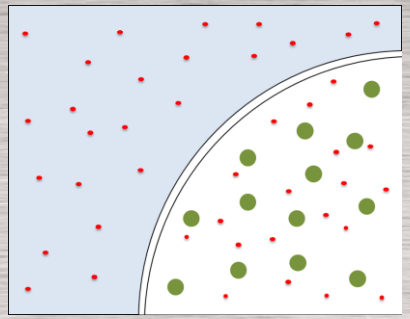
PTIP and the kinetics of cellular inhibitor accumulation

			Cellular uptake parameters			
Transporter	Inhibitor	PTIP (fold)	Time to steady state (min)	$K_{p,passive}$	PS_{inf} (µL/min/10^6 cells)	$K_{p,passive}$ / PS_{inf}
OCT1	Ledipasvir	>594	>180	123	0.171	719.30
OATP1B1	Venetoclax	>203	>180	263	0.92	285.87
OATP1B1	Cyclosporin A	5.88	120	259	0.92	281.52
OATP1B1	Saquinavir	3.17	60	416	10.4	40.00
OAT3	Benzbromarone	4.96	60	333	8.29	40.17
OAT1	Benzbromarone	1.82	15	396	11.1	35.68
OAT3	Valsartan	1.4	3	1.33	5.59	0.24
OCT2	Trimethoprim	1.28	1	4.85	9.2	0.53

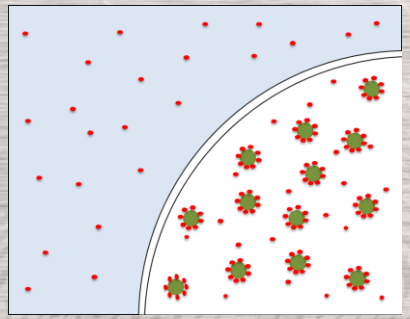




What do these data tell us about the mechanism?



$f_{u,\,IC} = 1$
$K_{p,passive} = 1$



$f_{u,\,IC} << 1$
$K_{p,passive} >> 1$

PS_{inf} ~ speed of uptake

High protein binding
(~ lipophilicity)

Slow passive diffusion
(~ molecular weight)

+ +

Assay duration << **TTSS**

IC_{50} w/o preinc > IC_{50} w/ preinc

PTIP

SOLVO® BIOTECHNOLOGY A CHARLES RIVER COMPANY

Conclusions of Topic #2

- PTIP affects SLC transporters other than OATP1B1/3 (OCTs > MATEs; OATs?)
- PTIP may influence DDI risk predictions → assay-specific cutoff should be determined with preincubation
- PTIP depends on molecular properties of the inhibitor (MW, $LogD_{7.4}$) as well as assay conditions (duration, BSA?)
- TTSS may exceed 3 hours → extension of the minimum recommended 30 minutes may need to be considered

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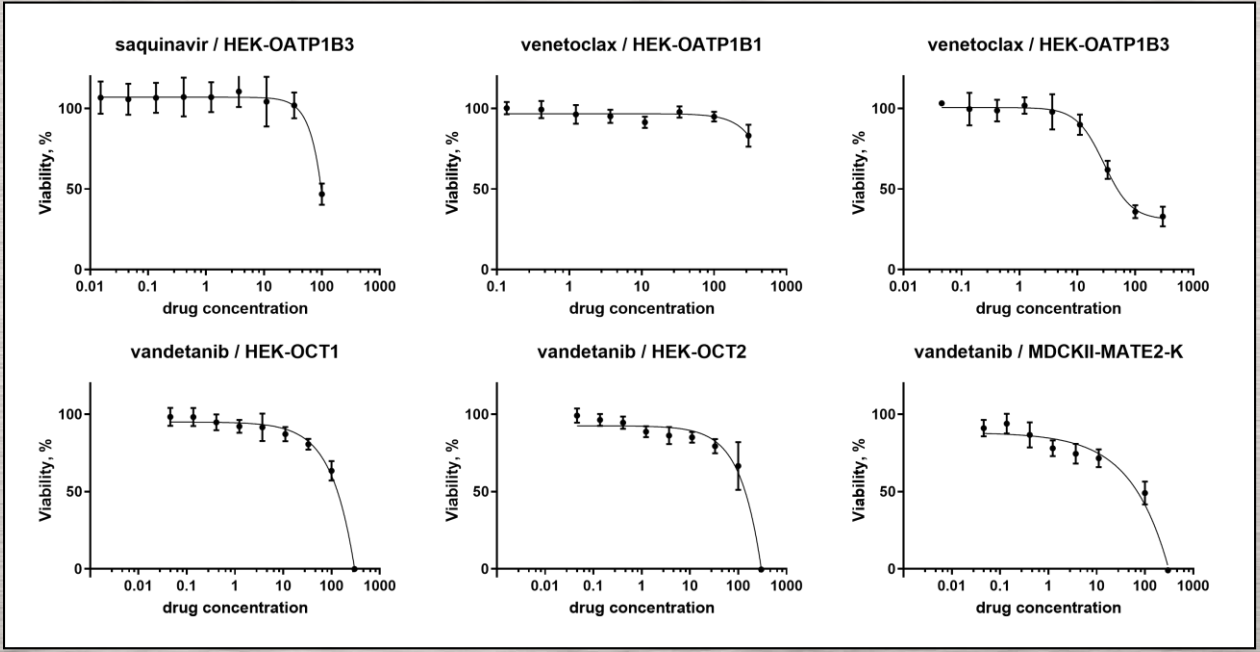
Thank you!

Supplemental slides

Toxicity does not explain PTIP

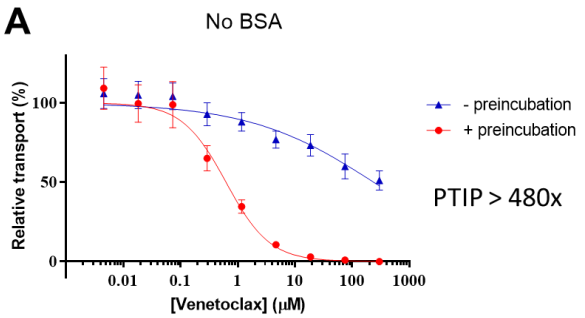
Inhibitor	Cell line	Conc. (µM)	Viability (% of control)
Cyclosporin A	HEK-OATP1B1	10	96.6 ± 4.8
	HEK-OATP1B3	10	102.2 ± 3.6
Daclatasvir	HEK-OCT1	50	106.3 ± 6.5
	HEK-OCT2	50	96.2 ± 4.6
Dolutegravir	HEK-OCT2	300	103.2 ± 4.3
Irinotecan	HEK-OCT1	50	102.8 ± 3.3
Isavuconazole	HEK-OCT1	300	95.0 ± 6.7
	HEK-OCT2	300	**32.2 ± 2.8**
Ledipasvir	HEK-OCT1	300	110.2 ± 8.3
	HEK-OCT2	300	111.1 ± 7.3
Saquinavir	HEK-OATP1B1	100	**55.2 ± 11.9**
	HEK-OATP1B3	100	**64.3 ± 6.3**
	HEK-OCT1	100	96.5 ± 7.8
Vandetanib	HEK-OCT1	300	**0.1 ± 0.1**
	HEK-OCT2	300	**0.2 ± 0.1**
	MDCKII-MATE2-K	300	**0.4 ± 0.1**
Venetoclax	HEK-OATP1B1	100	**89.6 ± 4.1**
	HEK-OATP1B3	100	**60.2 ± 4.1**
Verapamil	HEK-OCT1	100	**88.7 ± 5.8**



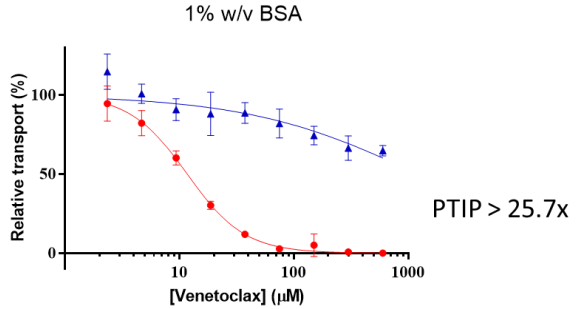
Protein binding only partly explains PTIP

Venetoclax / OATP1B1

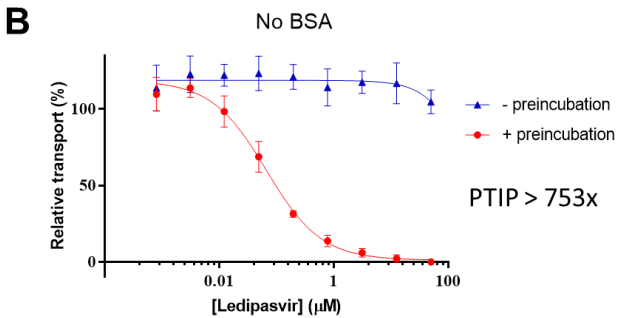
Ledipasvir / OCT1



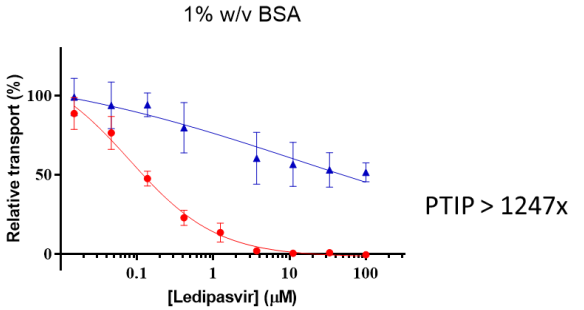
	- preincubation	+ preincubation
IC50	>300	0.6246



	- preincubation	+ preincubation
IC50	>300	11.69



	- preincubation	+ preincubation
IC50	>50	0.06642



	- preincubation	+ preincubation
IC50	>100	0.08017