SSX 3rd Annual Conference (Oct 11, 2018)

In vitro substrate-dependent inhibition of OATP1B1 and its impact on DDI prediction

Yoshitane Nozaki, PhD

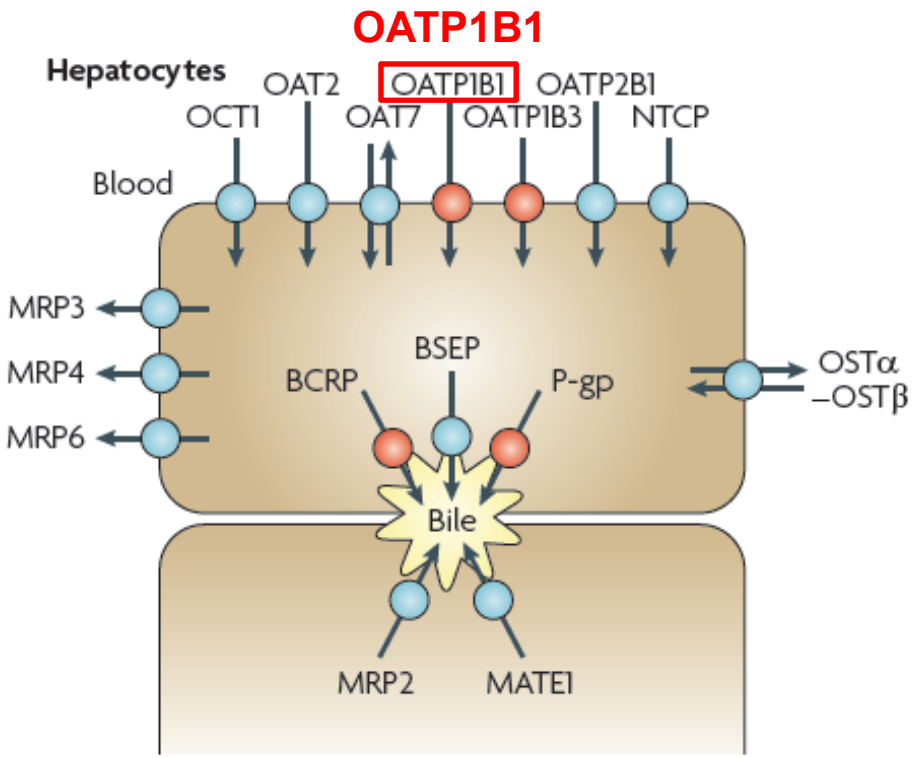
DMPK Tsukuba

Eisai Co., Ltd.

hhc

human health care

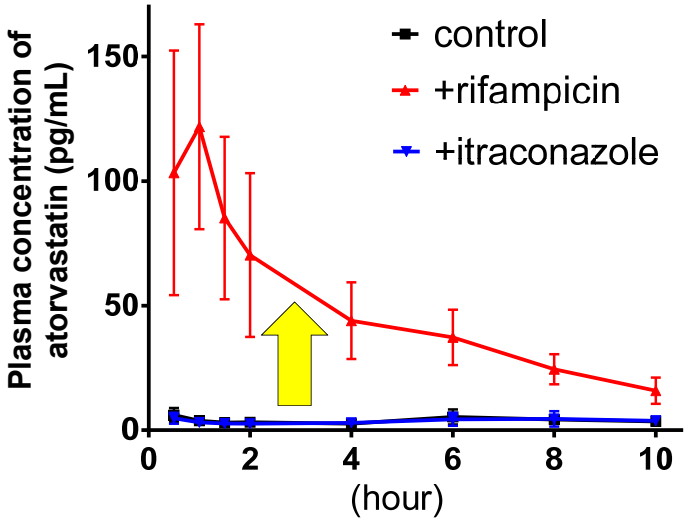
Organic Anion Transporting Polypeptide (OATP) 1B1



The International Transporter Consortium. *Nat Rev Drug Discov* 9:215-36 (2010)

Clinical DDI

Atorvastatin (OATP + CYP3A substrate)



	Control	+ Rifampin	+ Itraconazole
$AUC_{(0\text{-}10h)}$ (pg*h/mL)	38.5 ± 17.5	**439 ± 134*** **	36.0 ± 19.2

Maeda et al. *Clin Pharmacol Ther* 90: 575-81 (2011)

- OATP1B1 is involved in the hepatic uptake of various anionic drugs (eg. statins, ARBs).
- Clinically-relevant DDIs are caused by inhibition of OATP1B1. Higher exposure to OATP1B1 substrate drugs may lead to severe adverse effects (eg, rhabdomyolysis by statins).

In vitro OATP1B1 inhibition assay is routinely running in pharmaceutical companies to identify drug candidates with no or low risk of DDI perpetrator.

Risk assessment for DDIs (transporter inhibition)

Static model

$$R = 1 + \frac{[I]}{K_i}$$

- [I]: Inhibitor conc.
- K_i: Inhibition constant

$$R = 1 + \frac{[I]_{gut}}{IC_{50}} \geq 11$$

P-gp, BCRP (intestine)
$[I]_{gut}$: Molar dose/250 mL.

$$R = 1 + \frac{[I]_{u,inlet,max}}{K_i} \geq 1.1$$

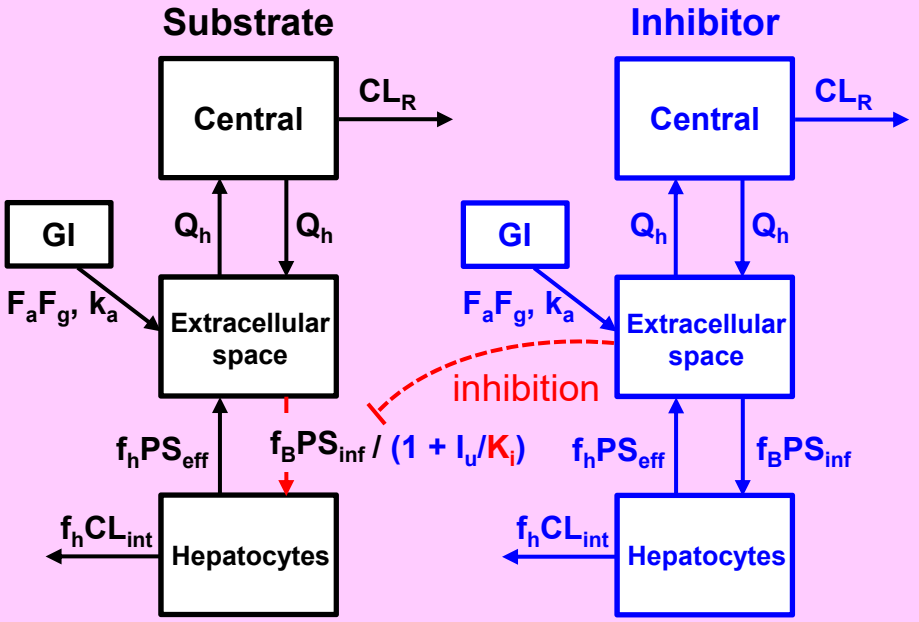
OATP1B1 & 1B3 (liver)
$[I]_{u,inlet,max}$: Estimated maximum inhibitor unbound conc. at the inlet to the liver.

$$R = 1 + \frac{C_{max,u}}{K_i} \geq \begin{matrix} 1.1\ \text{(OAT/OCT)} \\ 1.02\ \text{(MATE)} \end{matrix}$$

OAT1, OAT3, OCT2, MATE1, MATE2-K (kidney)
$C_{max,u}$: Maximum unbound plasma conc.

- ✓ Easy-to-use approach.
- ✓ Inhibitor concentration assumed to be constant.
- ✓ This model could overestimate DDI risk, but is helpful to avoid false-negative prediction.

Dynamic model



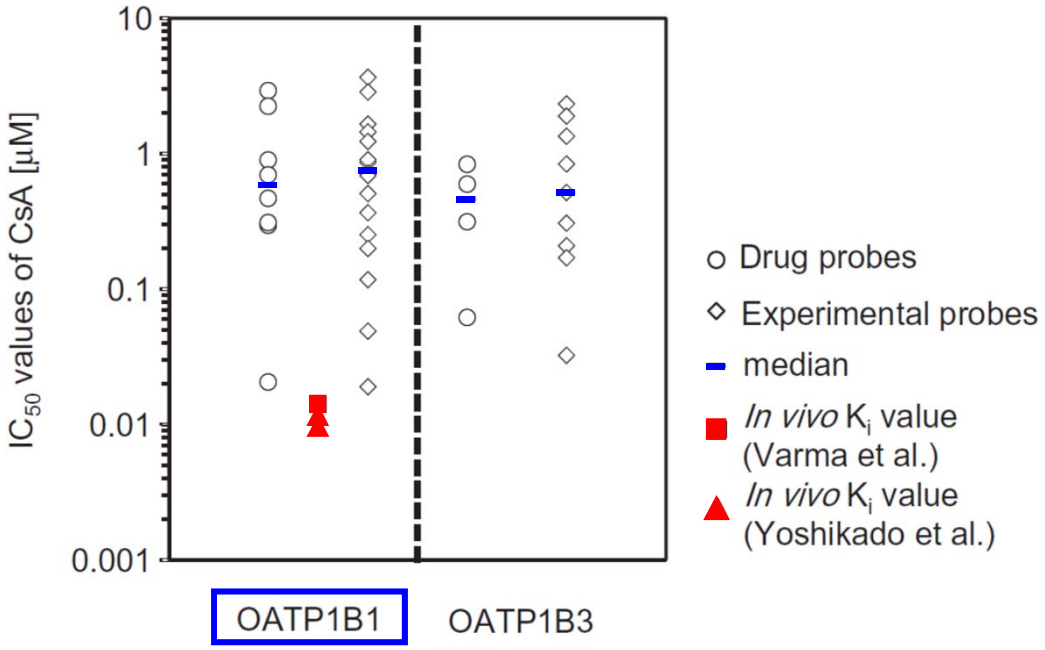
- ✓ Need to develop PK models for substrate and inhibitor.
- ✓ More quantitative prediction by considering time-profiles of substrate and inhibitor conc.

- In both models, K_i (or IC_{50}) value is a key parameter for DDI risk assessment.
- Accurate estimation of K_i (or IC_{50}) values from in vitro experiments is the critical step to achieve quantitative DDI prediction.

Variability in reported IC_{50} values for OATP1B1

Reported IC_{50} values of cyclosporine A (CsA) for OATP1B1

Shitara and Sugiyama. *Pharmacol Ther* 177: 67-80 (2017)

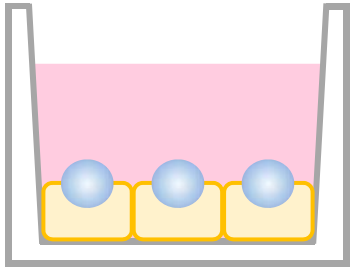


- In vitro probe substrates were classified into drug probes (eg, statins) and experimental probes (eg, estrone-3-sulfate).
- In either case, reported IC_{50} values showed >100-fold variability.

Outcomes of DDI risk assessment may be affected by large variability in IC_{50} values.

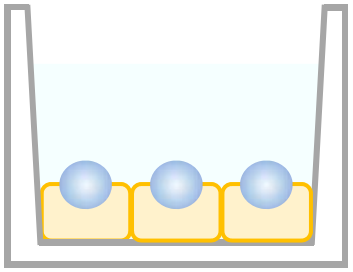
In vitro inhibition assay (uptake transporters)

Transporter-transfected cells
(eg, OATP1B1-HEK)



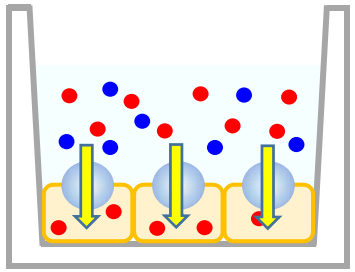
Cell seeding & culture

Mock cells
(negative control)

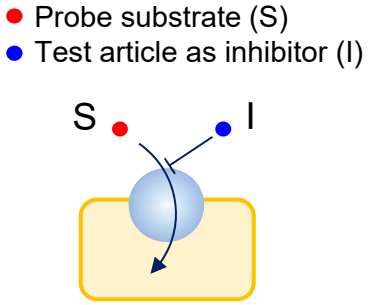


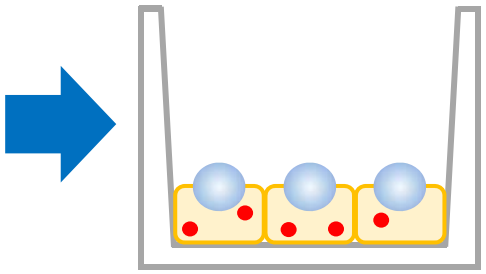
Pre-incubation

Probe substrate and test article (as inhibitor)
are simultaneously added to cells.
(assuming cis-inhibition)



Uptake reaction



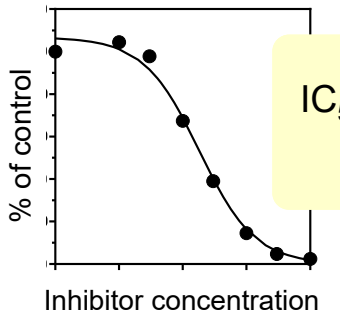


Buffer aspiration & wash

Sample treatment

- Extraction and quantification of probe substrate
- Protein assay
- Data analysis

$$\% \text{ of control} = \frac{100}{1+[I]/IC_{50}}$$

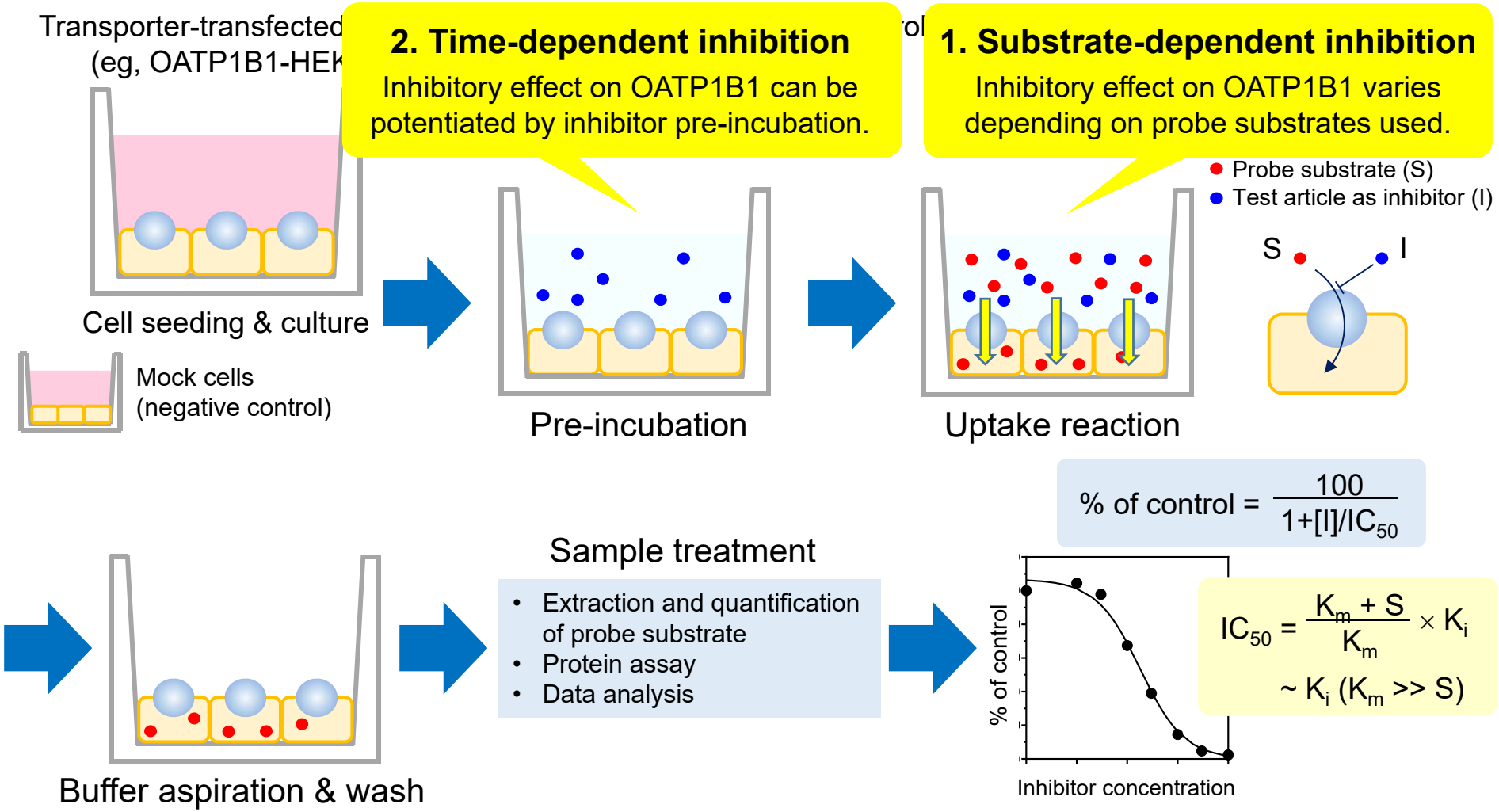


$$IC_{50} = \frac{K_m + S}{K_m} \times K_i$$

$$\sim K_i \ (K_m >> S)$$

- Determine uptake of probe substrate in the presence or absence of test article (as inhibitor)
- Calculate IC_{50} value from concentration-dependent decrease in transporter-mediated uptake.
- IC_{50} approximates K_i when probe substrate conc. << K_m (assuming competitive or non-competitive inhibition).

In vitro inhibition assay for uptake transporters



1. **Substrate-dependent inhibition** – Inhibitory effect of inhibitors on OATP1B1 greatly varied depending on the probe substrates used for in vitro assay.
2. **Time-dependent inhibition (pre-incubation effect)** – By pre-incubating cells with an inhibitor, the inhibitory effect on OATP1B1 could be potentiated.

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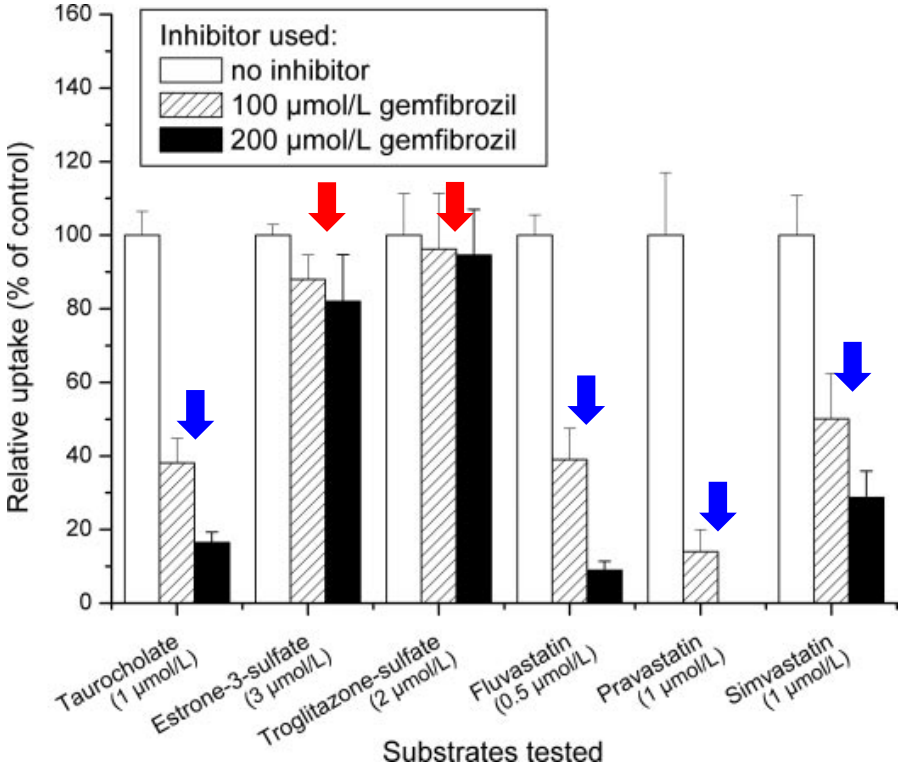
Substrate-dependent inhibition of OATP1B1

Inhibitory effect of gemfibrozil (GEM) on OATP1B1 in vitro



Noé J et al. *Drug Metab Dispos* 35:1308-14 (2007)

OATP1B1 substrates inhibited by GEM	OATP1B1 substrates NOT inhibited by GEM
Taurocholate Fluvastatin Pravastatin Simvastatin	Estrone-3-sulfate Troglitazone-sulfate

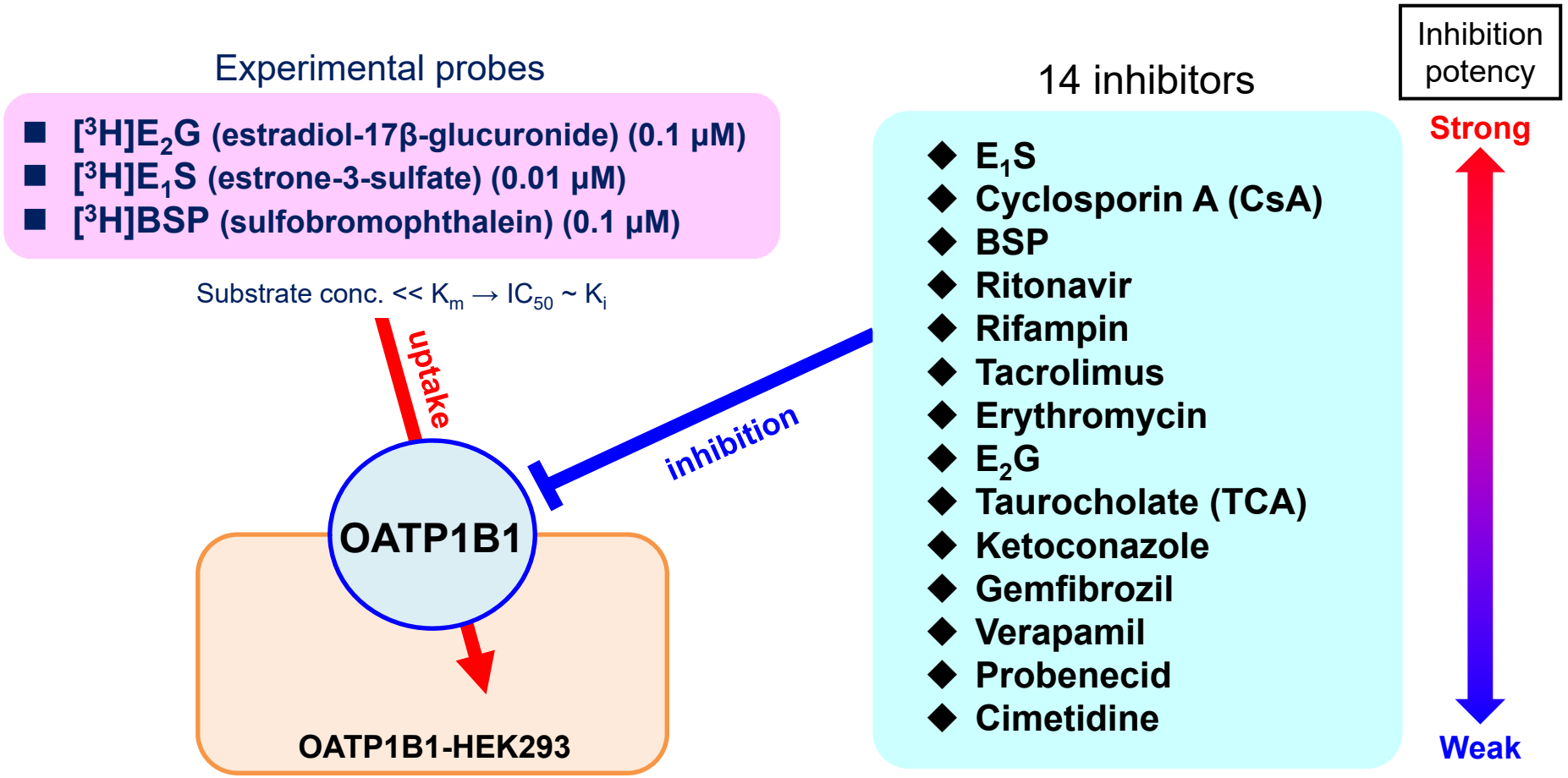
In vitro inhibitory effect of GEM on OATP1B1 varied greatly, depending on the probe substrates selected.

"Substrate-dependent Inhibition"

- **In DDI risk assessment, false-negative prediction should be avoided.**
- **Probe substrates that can offer lower (conservative) K_i values should be used in OATP1B1 inhibition assay.**

Substrate-dependent inhibition of OATP1B1 – Experimental probes –

- Due to the high detection sensitivity and simple quantification, radiolabeled experimental probes have been widely used in in vitro OATP1B1 inhibition assays.
- Three typical experimental probes (E_2G, E_1S and BSP) were tested.



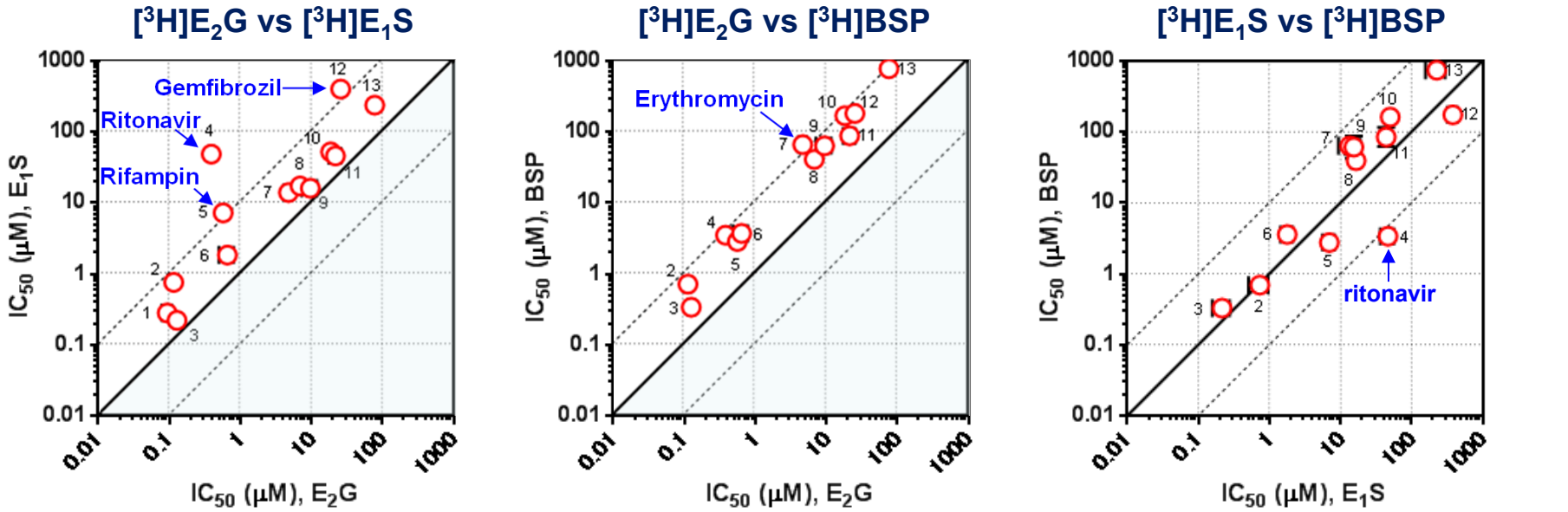
(Radioactivity taken up by cells was quantified by liquid scintillation counting.)

(Compound set that covered a wide range of inhibition potency was selected.)

Substrate-dependent inhibition of OATP1B1 – Experimental probes –

Comparison of K_i values of test inhibitors on OATP1B1 between 3 experimental probes

Izumi, Nozaki, et al. *Drug Metab Dispos* 41: 1859-1866 (2013)



Inhibitors: 1, E_1S; 2, CsA; 3, BSP; 4, ritonavir; 5, rifampin; 6, tacrolimus; 7, erythromycin; 8, E_2G; 9, ketoconazole; 10, TCA; 11, verapamil; 12, gemfibrozil; 13, probenecid.

- In some inhibitors (eg, Ritonarir, Gembibrozil, Rifampin), the K_i values for OATP1B1 varied by 10 ~ 100 fold (117 fold for Ritonavir), depending on the substrates used.
- Of the 3 substrates, [^{3}H]E_2G offered the lowest K_i values for all inhibitors examined.

Use of [^{3}H]E_2G as an in vitro probe may help mitigate the risk of false-negative DDI prediction cased by substrate-dependent K_i variability.

Substrate-dependent inhibition of OATP1B1
– Drug probes –

To further understand substrate-dependency of OATP1B1 inhibition, 12 drug probes - 3 inhibitors combinations were tested.

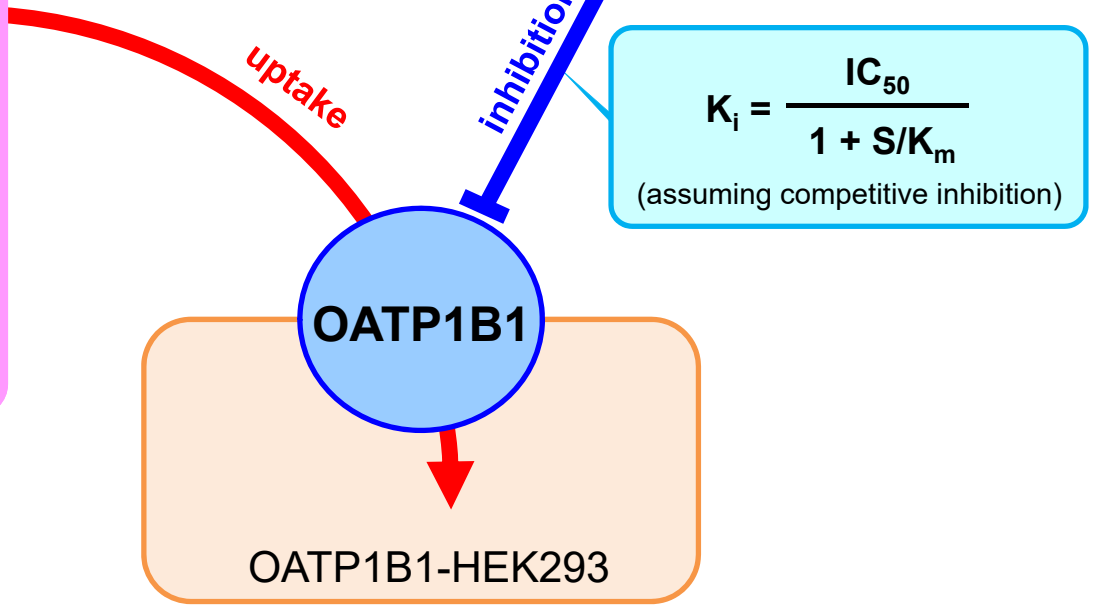
OATP1B substrate drugs

- **Pitavastatin (0.1 μmol/L)**
- **Atorvastatin (0.1 μmol/L)**
- **Fluvastatin (1 μmol/L)**
- **Rosuvastatin (1 μmol/L)**
- **Pravastatin (10 μmol/L)**
- **Repaglinide (0.1 μmol/L)**
- **Nateglinide (1 μmol/L)**
- **Glibenclamide (0.1 μmol/L)**
- **Bosentan (0.1 μmol/L)**
- **Valsartan (1 μmol/L)**
- **Torasemide (1 μmol/L)**
- **Fexofenadine (1 μmol/L)**

Substrate conc. < K_m

Inhibitors

- **Cyclosporin A (CsA) (strong)**
- **Rifampin (RIF) (strong)**
- **Gemfibrozil (GEM) (weak)**



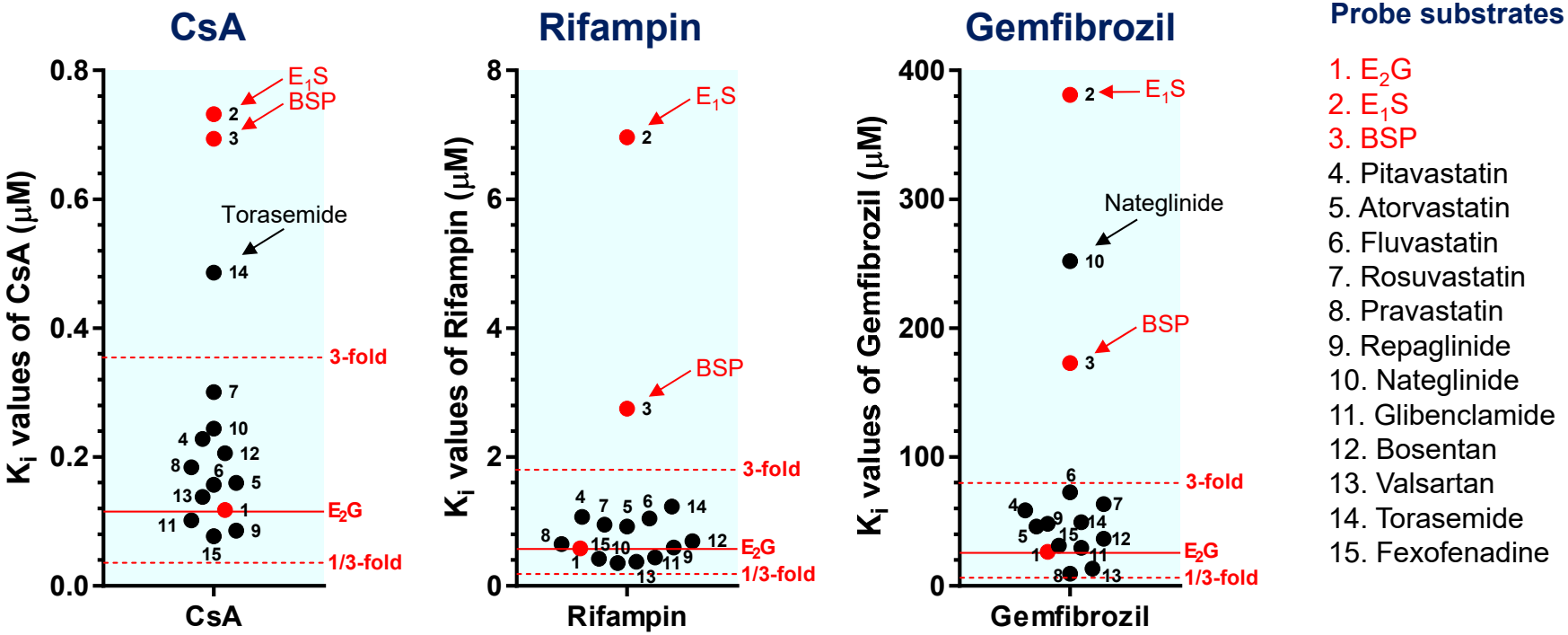
$$K_i = \frac{IC_{50}}{1 + S/K_m}$$

(assuming competitive inhibition)

(The compounds taken up by cells were quantified by LC-MS/MS.)

Substrate-dependent inhibition of OATP1B1 – Drug probes –

Izumi, Nozaki et al. *Drug Metab Dispos* 43: 235-247 (2015)



Probe substrates

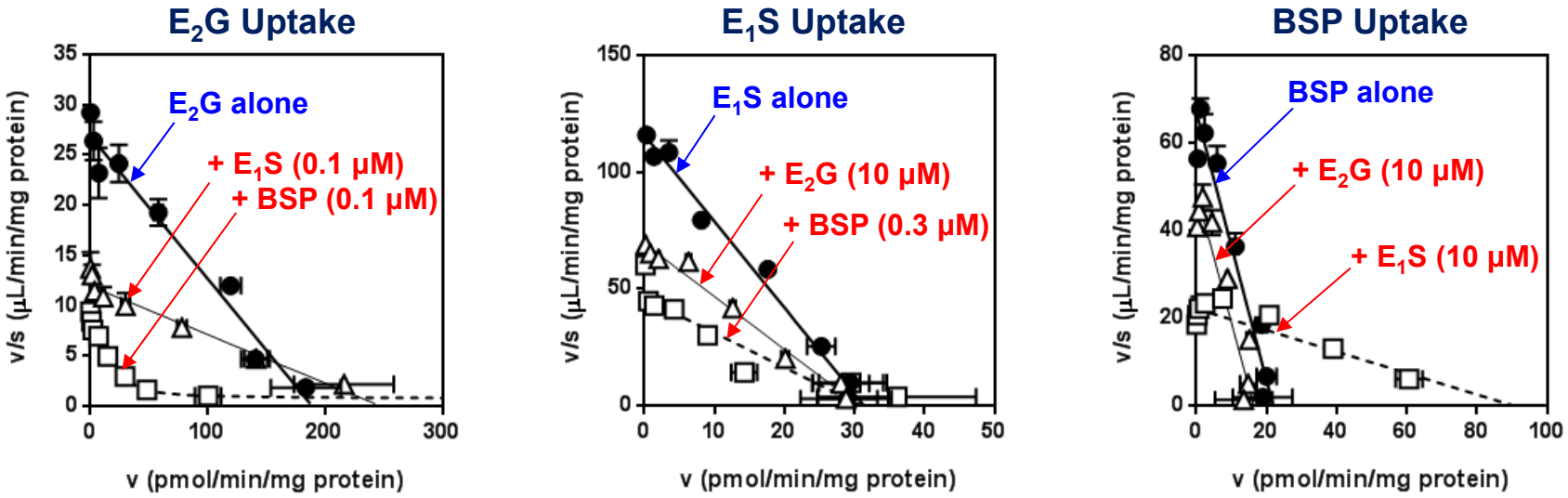
1. E_2G
2. E_1S
3. BSP
4. Pitavastatin
5. Atorvastatin
6. Fluvastatin
7. Rosuvastatin
8. Pravastatin
9. Repaglinide
10. Nateglinide
11. Glibenclamide
12. Bosentan
13. Valsartan
14. Torasemide
15. Fexofenadine

- $[^3H]E_1S$ (typical experimental probe) showed higher K_i values for 3 inhibitors.
- $[^3H]E_2G$ and the majority of drug probes (except for torasemide & nateglinide) gave similar K_i values (within 3-fold) and covered lower limit of K_i values range.

In OATP1B1 inhibition assays, $[^3H]E_2G$ or drug probes should be used, so that the risk of false-negative DDI prediction potentially cased by substrate-dependent K_i variability could be reduced.

Mechanism of substrate-dependent inhibition of OATP1B1

Mutual inhibition study for E_2G, E_1S and BSP to understand the mode of interaction



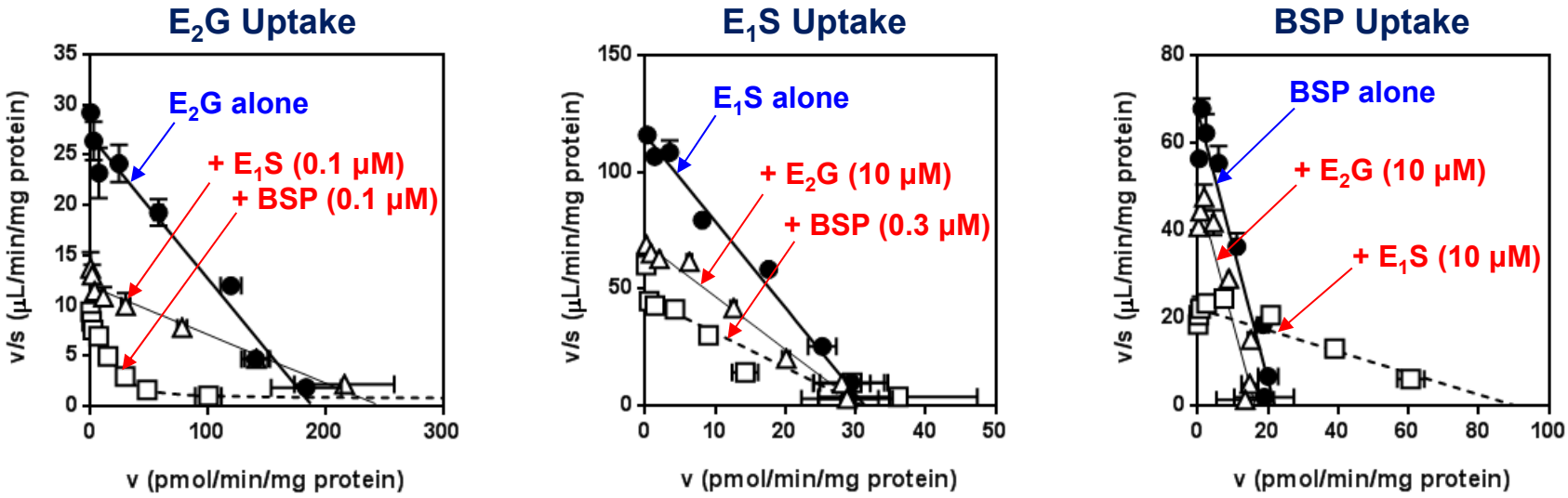
Inhibitors	Substrate						
	[^{3}H]E_2G			[^{3}H]E_1S		[^{3}H]BSP	
	K_m	V_{max}	P_{dif}	K_m	V_{max}	K_m	V_{max}
No inhibitor	8.17 ± 2.28	250 ± 89	-	0.236 ± 0.054	36.4 ± 10.3	0.280 ± 0.041	20.8 ± 1.0
E_2G	-	-	-	0.488 ± 0.072*	35.7 ± 8.3	0.361 ± 0.069	18.5 ± 1.9
E_1S	18.7 ± 2.2**	251 ± 22	-	-	-	4.78 ± 0.65**	111 ± 18**
BSP	5.80 ± 2.53	55.8 ± 24.7**	0.667 ± 0.642	0.677 ± 0.112*	34.3 ± 11.7		-

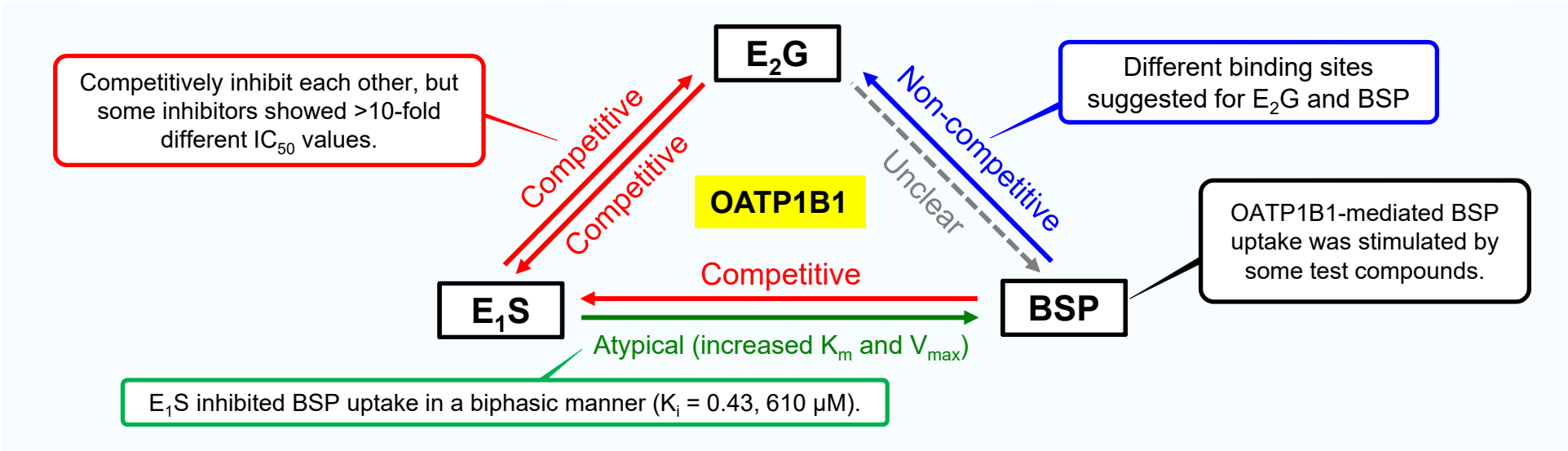
*P<0.05; **P<0.01 compared with parameters determined without any inhibitors

Izumi, Nozaki et al. *Drug Metab Dispos* 41: 1859-1866 (2013)

Mechanism of substrate-dependent inhibition of OATP1B1

Mutual inhibition study for E_2G, E_1S and BSP to understand the mode of interaction





These findings can not be accounted for by single substrate binding site on OATP1B1

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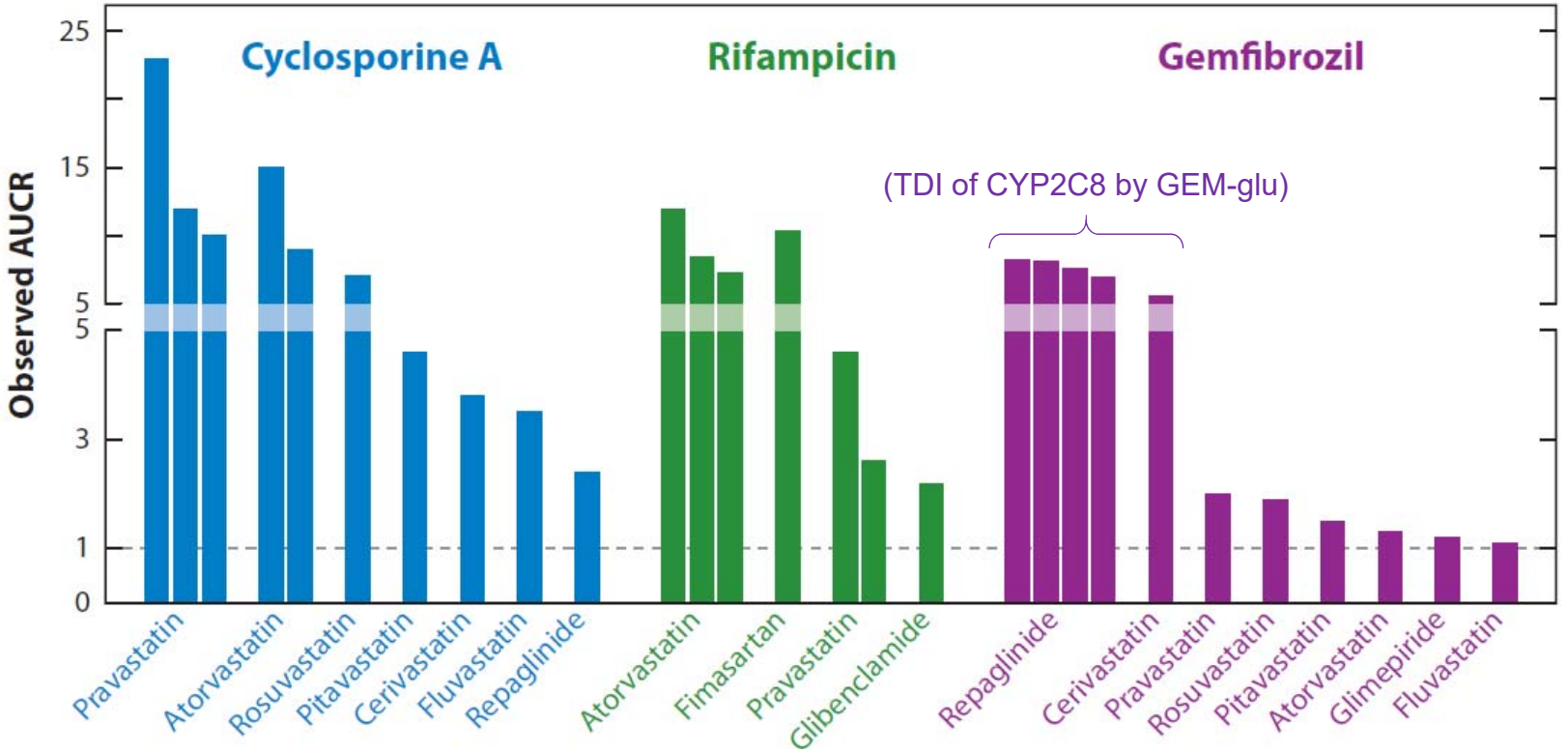
Impact of substrate-dependent K_i variability on DDI risk assessment with static models

Clinical DDIs with OATP substrate drugs



Yoshida et al. *Annu Rev Pharmacol Toxicol* 53: 581-612 (2013)

CsA, RIF and GEM are clinically-relevant OATP inhibitors (AUCR ≥ ~2).

Impact of substrate-dependent K_i variability on DDI risk assessment with static models

According to the regulatory DDI guidances, R values of CsA, rifampin and gemfibrozil were calculated using their in vitro K_i values on OATP1B1 obtained from various probes.

$[I]_{u,inlet,max}$

Clinical PK parameters (CsA, Rifampin, Gemfibrozil)

Inhibitors	Dose	f_u	C_{max}	$[I]_{u,inlet,max}$
	(mg)	*(%)*	*(μM)*	*(μM)*
CsA	200[a]	11[b]	0.95[a]	1.2
Rifampin	600[c]	15[d]	23[e]	10
Gemfibrozil (GEM-glu)	600[c]	0.65[f,h] (0.115)	100[g] (70.1)	1.6 (NA)

[a]Mück et al., 1999; [b]Lemaire and Tillement, 1982; [c]Yoshida et al., 2012; [d]Burman et al., 2001; [e]Maeda et al., 2011; [f]Shitara et al., 2004; [g]Okerholm et al., 1976

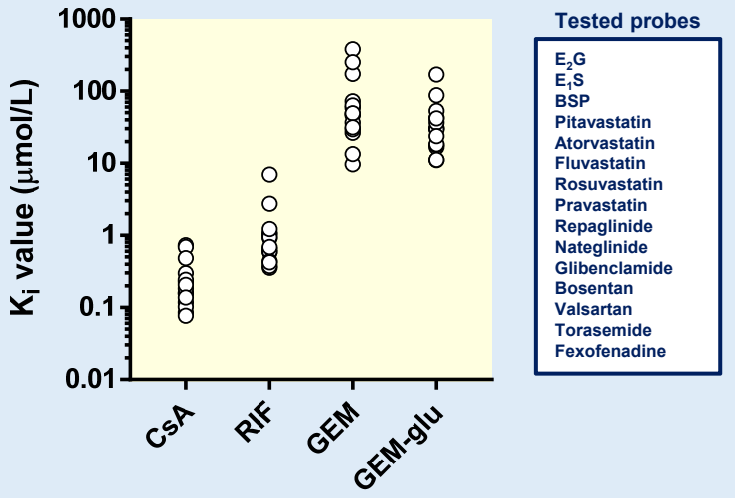
[h]As the f_u of gemfibrozil was less than 1%, the f_u was assumed to be 1%.

$$[I]_{u,inlet,max} = f_u \times \left(I_{max} + \frac{k_a \times F_a \times F_g \times Dose}{Q_h} \right)$$

(Assumption; R_B=1, k_a=0.1 min^{-1}, F_aF_g=1, Q_h=97L/h)

In vitro K_i for OATP1B1

K_i values of CsA, RIF, GEM & GEM-glu determined with various probe substrates



Static model (OATP1B1 inhibition)

$$R = 1 + \frac{[I]_{u,inlet,max}}{K_i}$$

How much R value variability can be produced by substrate-dependent OATP1B1 inhibition?

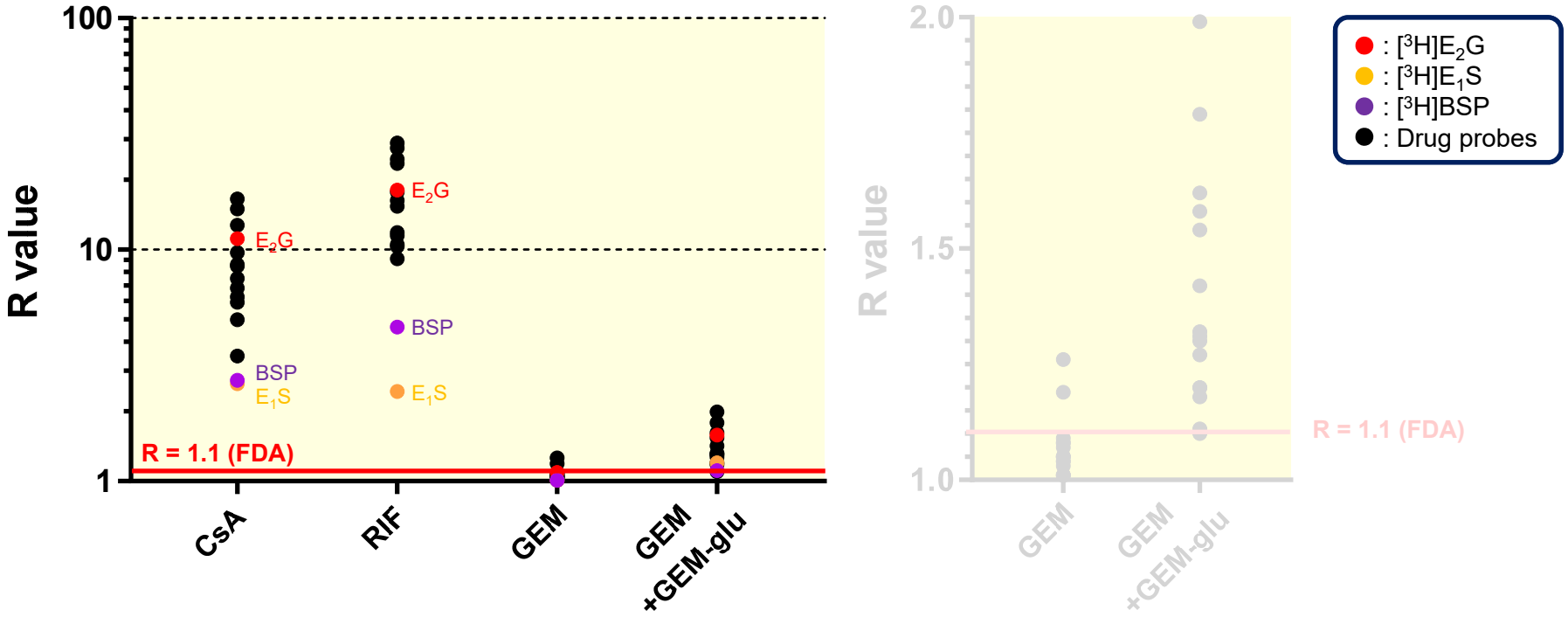
Impact of substrate-dependent K_i variability on DDI risk assessment with static models

Static model
(OATP1B1 inhibition)

$$R = 1 + \frac{[I]_{u,inlet,max}}{K_i}$$

R ≥ 1.1 (US & JP)
- Potential inhibitor. Clinical DDI study may be required to evaluate the inhibitory effect on OATPs in vivo.

R < 1.1 (US & JP)
- The risk to inhibit OATPs in vivo is very low. No need to perform clinical DDI study.



- **R values for CsA and RIF were ≥1.1 regardless of probe substrates. These inhibitors were correctly judged as potential OATP1B1 inhibitors in vivo.**
- **No impact on DDI risk assessment for strong OATP1B1 inhibitors.**

Impact of substrate-dependent K_i variability on DDI risk assessment with static models

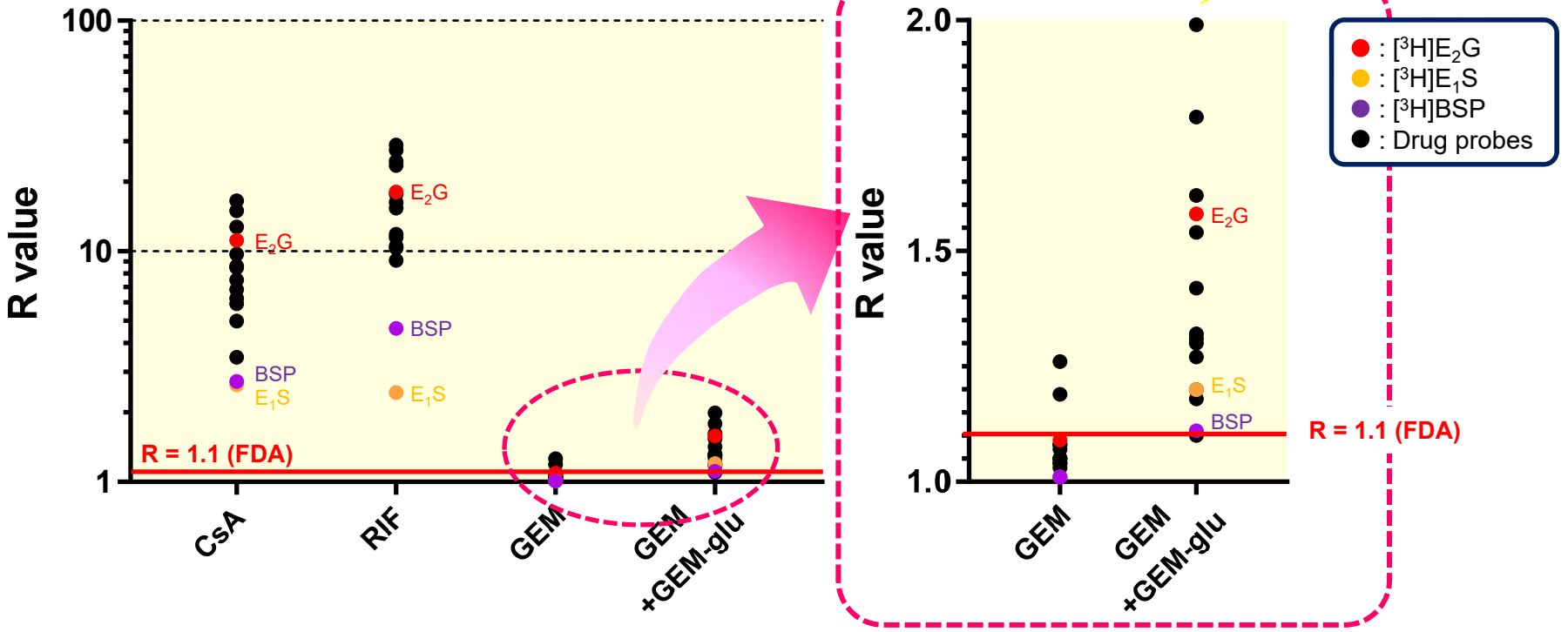
Static model
(OATP1B1 inhibition)

$$R = 1 + \frac{[I]_{u,inlet,max}}{K_i}$$

R ≥ 1.1 (US & JP)
- Potential inhibitor. Clinical DDI study may be required to evaluate the

R < 1.1 (US &
- The risk to inhi
perform clinical

$$R = 1 + \frac{[I]_{u,inlet,max,GEM}}{K_{i,GEM}} + \frac{C_{max,u,Gem\text{-}glu}}{K_{i,Gem\text{-}glu}}$$



- R values for GEM + GEM-glu were ≥1.1 regardless of probe substrates. However, some substrates (eg, E_1S and BSP) offered borderline values.
- To avoid potential false-negative prediction for GEM (weak-to-moderate inhibitor), sensitive probes such as E_2G should be used for in vitro assays.

Contents

1. Substrate-dependent inhibition of OATP1B1 in vitro
2. Impact of substrate-dependent K_i variability on DDI risk assessment with static model
3. Fluorescent substrates for OATP1B1
4. Time-dependent inhibition of OATP1B1 by cyclosporine A

Fluorescence-based OATP1B1 inhibition assay

- Fluorescence-based OATP1B1 inhibition assay can offer highly-sensitive, high-throughput method to screen out synthesized compounds with high DDI risk.
- Useful particularly at the early stage of drug development.

Many fluorescent substrates are reported for OATP1B1, however…

Reported fluorescent substrates of OATPs

Not commercially available

Commercially available

Fluorescent substrates	Labeled substances	Fluorophore	Transporters
CDCA-NBD	Bile acid	NBD[a]	OATP1B1 / 1B3, rat Oatp(s)
CA-NBD	Bile acid	NBD[a]	OATP1B1 / 1B3, rat Otap(s)
DCA-NBD	Bile acid	NBD[a]	OATP1B1 / 1B3
LCA-NBD	Bile acid	NBD[a]	OATP1B1 / 1B3
UDCA-NBD	Bile acid	NBD[a]	OATP1B1 / 1B3
CGamF	Bile acid	FL[b]	OATP1B1 / 1B3, rat Oatp(s)
CLF	Bile acid	FL[b]	OATP1B1 / 1B3
FMTX	Methotrexate	FL[b]	OATP1B1 / 1B3
Flutax-2	Paclitaxel	Og[c]	OATP1B3
FL	-	FL[b]	OATP1B1 / 1B3, rat Oatp(s)
8-FcA	cAMP	FL[b]	OATP1B1 / 1B3
Fluo-3	-	Fluo-3	OATP1B3
CDCF	-	CDCF	Rat Oatp(s)

a: Nitrobenzoxadiazole, b: Fluorescein, c: Oregon green

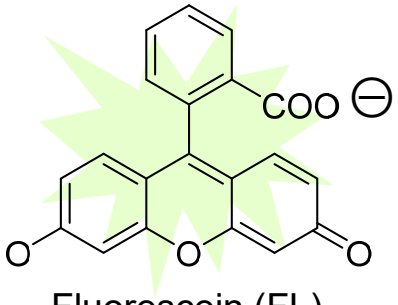
– Current issues –

- ✓ **Availability from commercial sources**
  (Need to be synthesized before use)
- ✓ **Low detection sensitivity**
  (low cellular accumulation and/or low fluorescence quantum yield)
- ✓ **Potential safety concern**
  (anticancer drug analogues)
- ✓ **High reagent cost**

– Our goal –

To find novel OATP1B1 fluorescent substrate(s) that is applicable to in vitro inhibition assay systems.

Identification of novel fluorescent substrates of OATP1B1



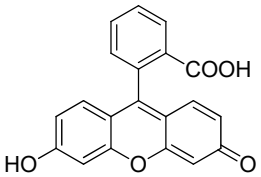
Fluorescein (FL)

Fluorescein and its derivatives

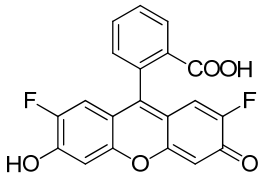
- ✓ Emit strong fluorescence.
- ✓ Negatively charged at pH 7.4.

Following FL and its derivatives were tested to find novel fluorescent substrates of OATP1B1

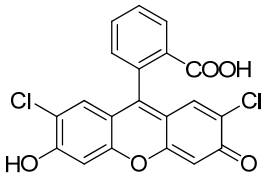
FL



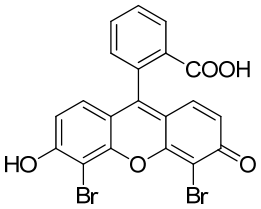
OG



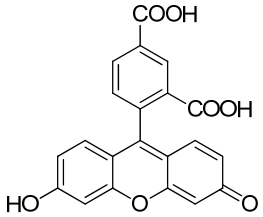
DCF



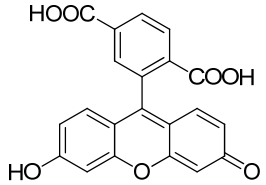
DBF



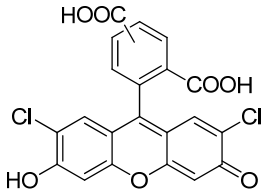
5-CF



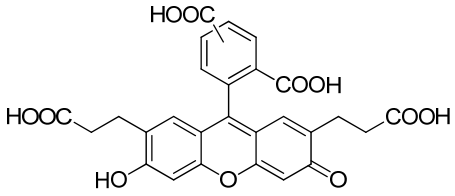
6-CF



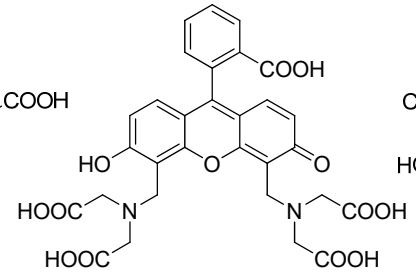
CDCF



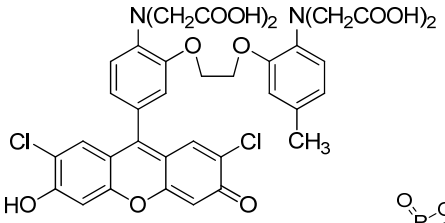
BCECF



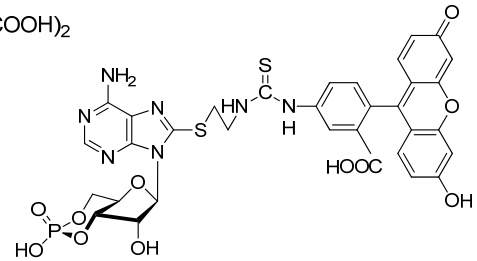
Calcein



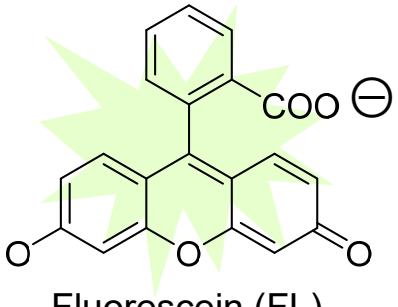
Fluo-3



8-FcA



Identification of novel fluorescent substrates of OATP1B1

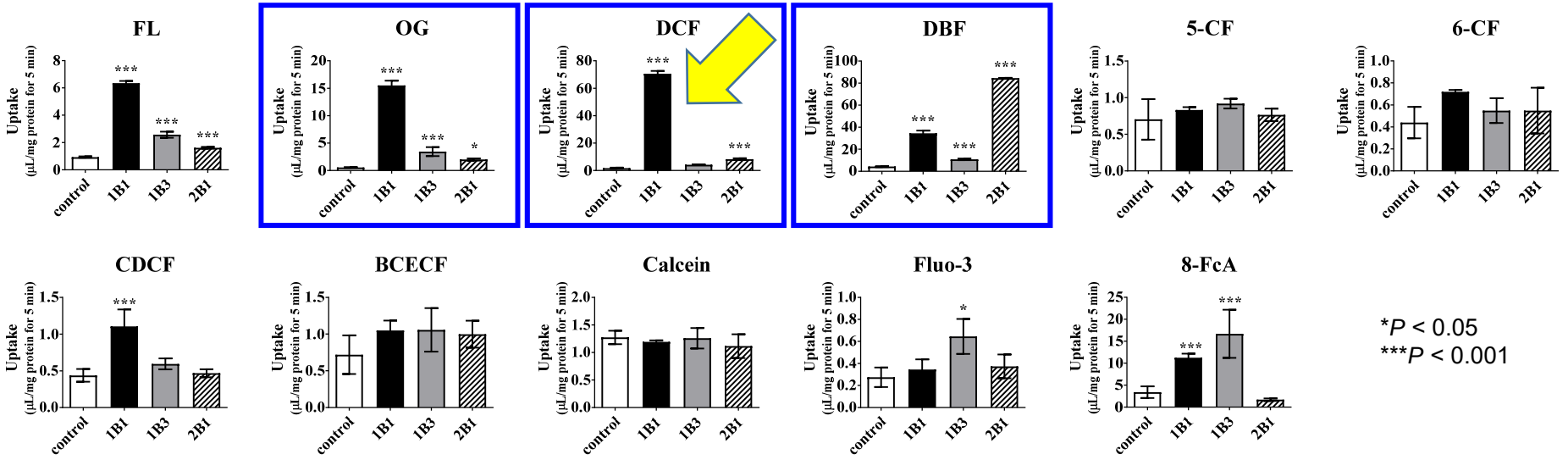


Fluorescein (FL)

Fluorescein and its derivatives

- ✓ Emit strong fluorescence.
- ✓ Negatively charged at pH 7.4.

Uptake of FL and its derivatives in OATP1B1-, OATP1B3- and OATP2B1-HEK293 cells



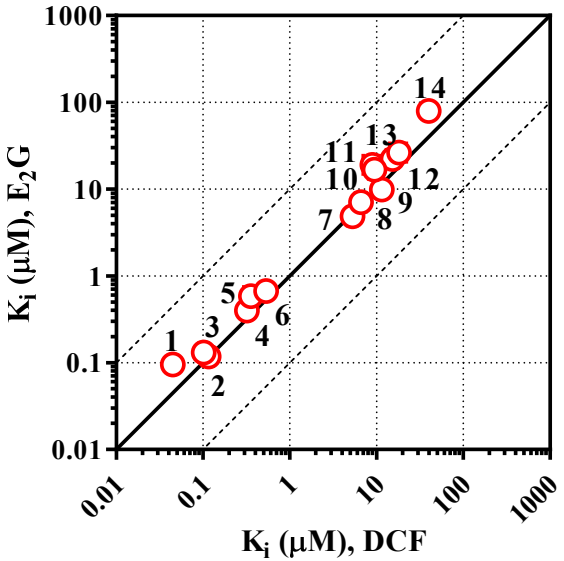
Izumi, Nozaki et al. *Mol. Pharmaceutics* 13:438-448 (2016)

- OG, DCF, and DBF were newly identified as fluorescent OATP1B1 substrates.
- DCF (dichlorofluorescein) showed the highest OATP1B1-mediated uptake of the FL derivatives examined.

Characterization of DCF as OATP1B1 probe substrate

Substrate-dependent inhibition

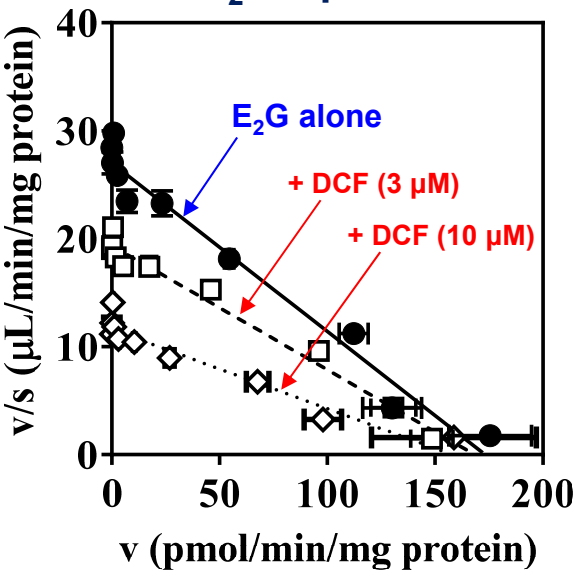
[^{3}H]E_2G vs DCF



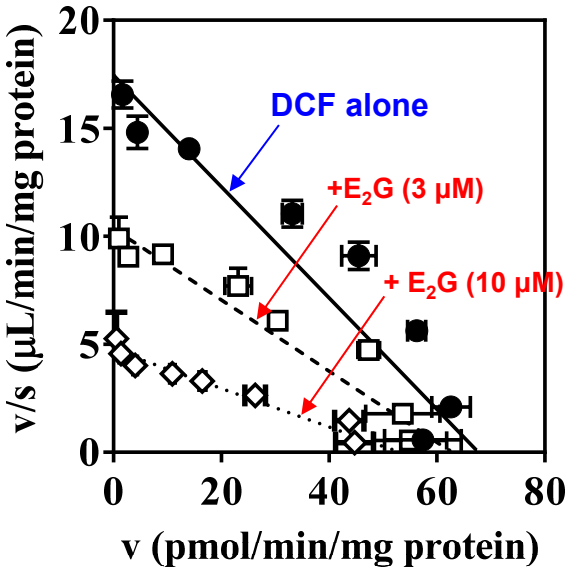
1.	E_1S	8.	E_2G
2.	CsA	9.	Ketoconazole
3.	BSP	10.	Gem-glu
4.	Ritonavir	11.	TCA
5.	Rifampin	12.	Verapamil
6.	Tacrolimus	13.	Gemfibrozil
7.	Erythromycin	14.	Probenecid

Mutual inhibition between E_2G and DCF

E_2G Uptake



DCF Uptake



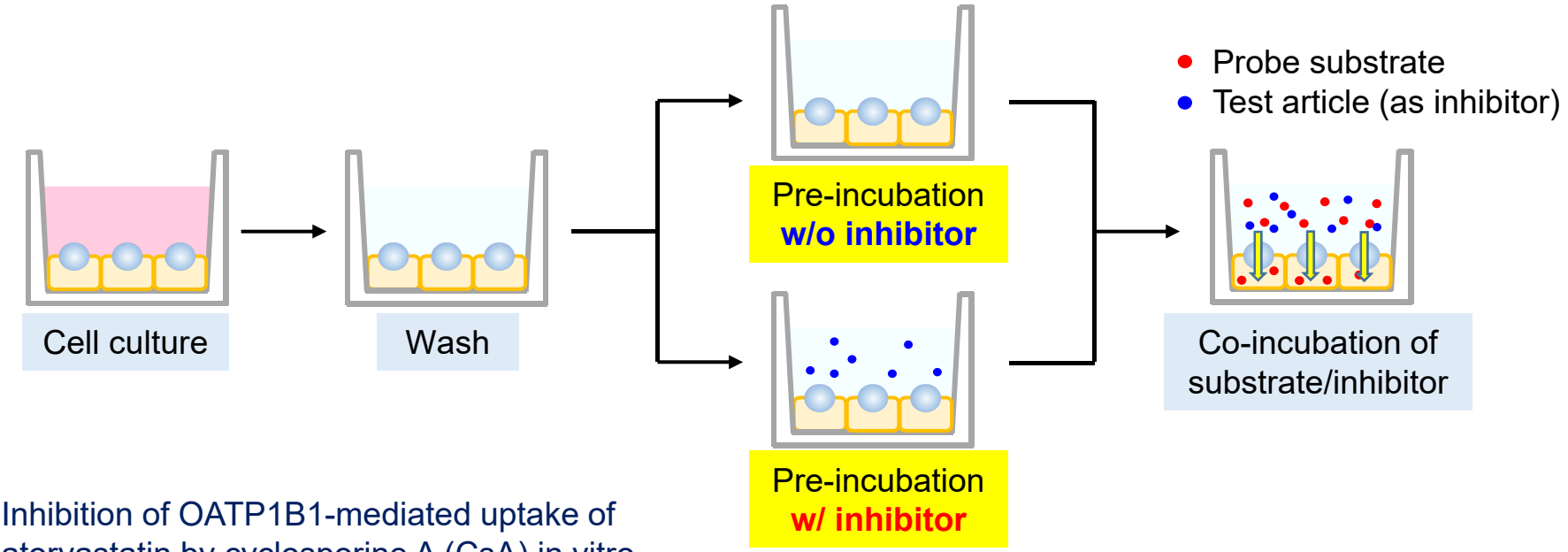
- DCF and E_2G provided similar K_i values for 14 inhibitors.
- DCF and E_2G competitively inhibited each other, suggesting they share the same binding site on OATP1B1.
- DCF can be used an alternative probe to E_2G.

Fluorescence-based OATP1B1 inhibition assay with DCF as a probe can be used as a highly-sensitive, high-throughput screening measures at the early stage of drug development.

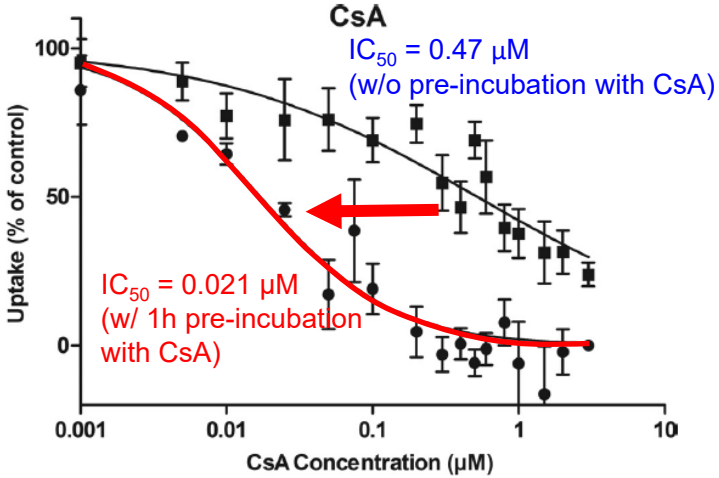
Contents

Time-dependent inhibition of OATP1B1 (Pre-incubation effect)

Typical study design to investigate time-dependency of transporter inhibition



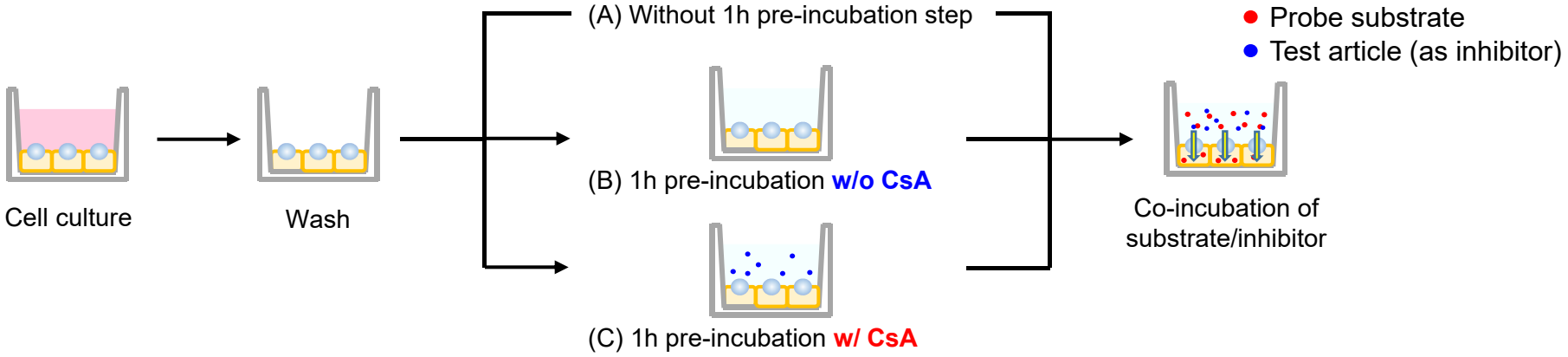
Inhibition of OATP1B1-mediated uptake of atorvastatin by cyclosporine A (CsA) in vitro

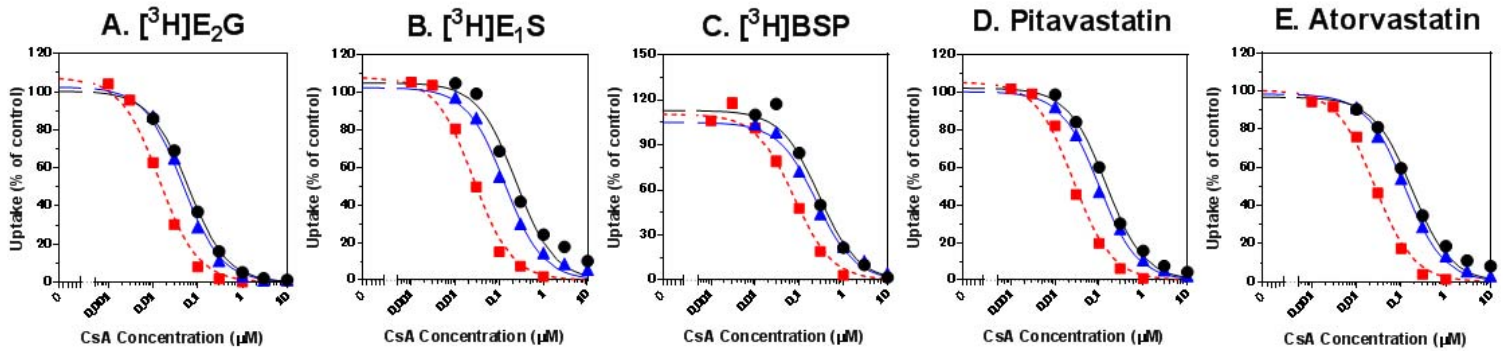


Amundsen et al. *Drug Metab Dispos* 38: 1499-1504 (2010)

- Assuming cis-inhibition, co-incubation of a probe substrate and an inhibitor has been employed in transporter inhibition assay.
- By pre-incubating cells with CsA before atorvastatin-CsA co-incubation, inhibitory effect of CsA on OATP1B1 was potentiated.

Time-dependent inhibition of OATP1B1 (Pre-incubation effect)





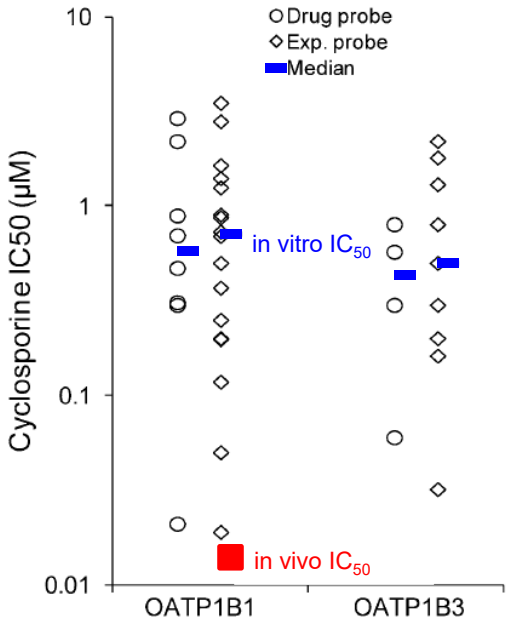
Substrates	K_i of CsA			Fold potentiation (C) vs (B)
	(A) No pre-incubation	(B) After 1-hour pre-incubation without CsA	(C) After 1-hour pre-incubation with CsA	
	(μM)	(μM)	(μM)	
E_2G	0.0620 ± 0.0142	0.0458 ± 0.0041	0.0139 ± 0.0066	3.3 fold
E_1S	0.253 ± 0.088	0.134 ± 0.017	0.0264 ± 0.0085	5.1 fold
BSP	0.283 ± 0.070	0.252 ± 0.057	0.0799 ± 0.0273	3.2 fold
Pitavastatin	0.138 ± 0.014	0.0985 ± 0.0360	0.0252 ± 0.0038	3.9 fold
Atorvastatin	0.156 ± 0.016	0.0986 ± 0.0250	0.0229 ± 0.0033	4.3 fold

Izumi, Nozaki et al. *Drug Metab Dispos* 43: 235-247 (2015)

By CsA pre-incubation, inhibitory effect of CsA on OATP1B1 was potentiated by 3 to 5 fold regardless of probe substrates.

Discrepancy between in vitro and in vivo IC_{50} values of CsA for OATP1B1

Inhibitor : CsA



Li et al. *Clin Pharmacokinet* 53: 659-678 (2014)

Transporters	Substrates	Pre-incubation time (min)	K_i or IC_{50} of CsA		Ratio
			Without Pre-incubation	With Pre-incubation	
			(μmol/L)	(μmol/L)	
OATP1B1[a]	Atorvastatin	60	0.47	0.021	22.4
OATP1B1[b]	$[^3H]E_2G$	60	0.0458	0.0139	3.3
	$[^3H]E_1S$		0.134	0.0264	5.1
	$[^3H]$BSP		0.252	0.0799	3.2
	Pitavastatin		0.0985	0.0252	3.9
	Atorvastatin		0.0986	0.0229	4.3
OATP1B1[c]	$[^3H]E_2G$	30	0.198	0.019	10.4
OATP1B3[c]	$[^3H]E_2G$		0.162	0.032	2.6

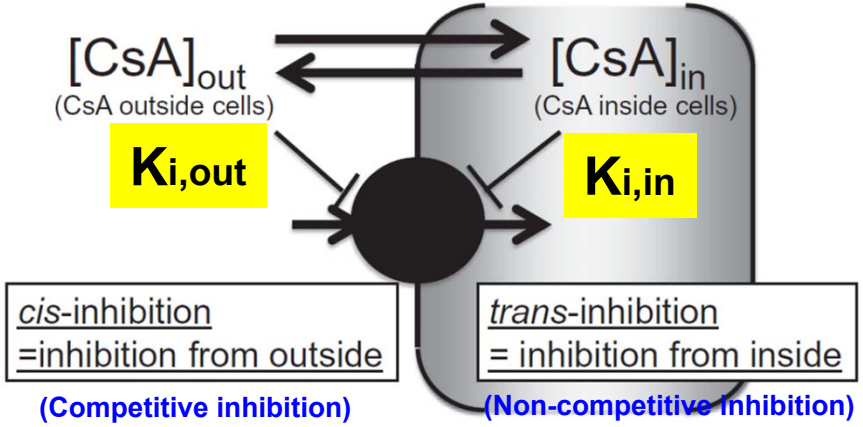
a) Amundsen et al. *Drug Metab Dispos* 38: 1499-1504 (2010)
b) Izumi et al. *Drug Metab Dispos* 43: 235-247 (2015)
c) Gertz et al. *Pharm Res* 30: 761-780 (2013)

- In vivo IC_{50} values estimated by PBPK modeling were much lower than in vitro IC_{50} values.
- By pre-incubating cells with CsA, the in vitro IC_{50} values were reduced to 14 ~ 80 nmol/L, which were similar to in vivo IC_{50} values.

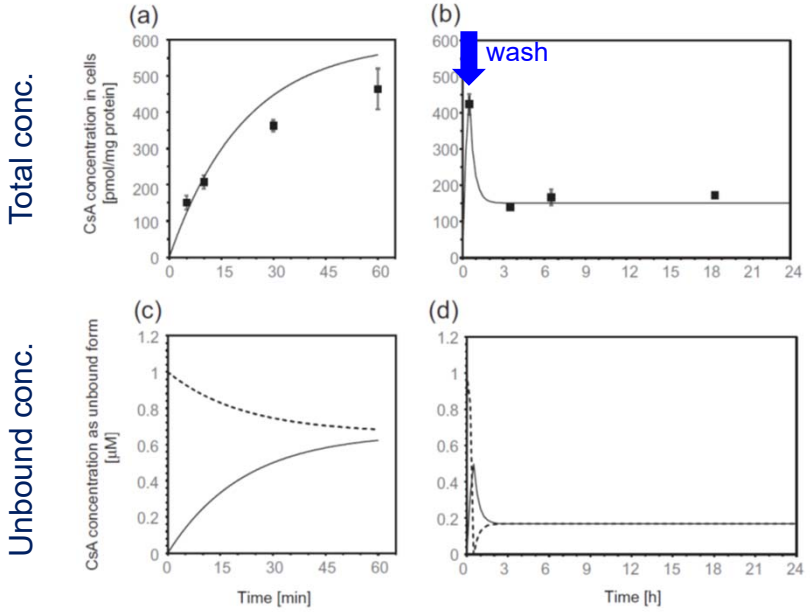
Inhibitor pre-incubation approach can offer conservative (lower) IC_{50} values for OATP1B1, which will be helpful to avoid false-negative DDI prediction and to estimate in vivo IC_{50} values.

Proposed mechanism of time-dependent OATP1B1 inhibition by CsA

- CsA inhibits OATP1B1 from both outside (cis-inhibition) and inside (trans-inhibition) of cells.
- Trans-inhibition potency is greater than cis-inhibition.
- CsA is slowly take up by the cells due to strong intracellular binding.



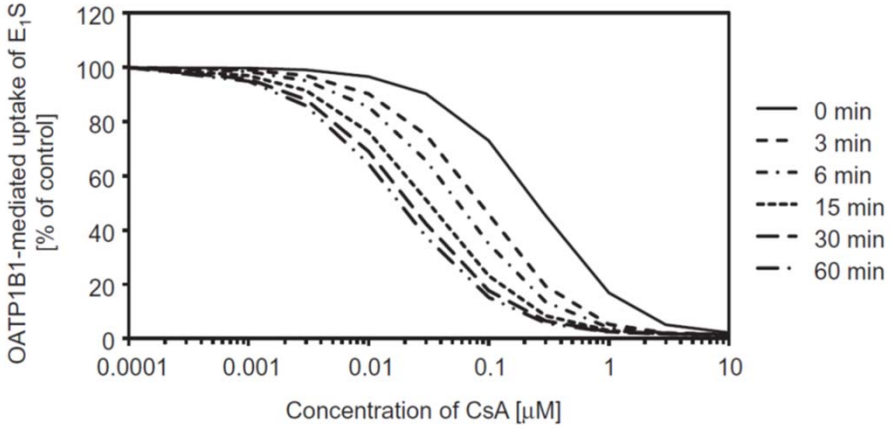
Intracellular and medium concentration of CsA



$C_{medium} << K_m$

$$\frac{dX_{cell}}{dt} = \frac{V_{max} / \left(1 + f_{u,i} \cdot I_{cell} / K_{i,in}\right)}{K_m \cdot \left(1 + I_{medium} / K_{i,out}\right) + C_{medium}} C_{medium}$$

$$\frac{dX_{cell}}{dt} = \frac{PS_{act}}{\left(1 + I_{medium} / K_{i,out}\right) \cdot \left(1 + f_{u,i} \cdot I_{cell} / K_{i,in}\right)} C_{medium}$$



Pre-incubation time-dependent potentiation of OATP1B1 inhibition by CsA was reproduced by this model.

Shitara and Sugiyama. *Pharmacol Ther* 177:67-80 (2017)

Time-dependent inhibition of transporters

Transporters	Inhibitors	
	Preincubation effect (time-dependent inhibition)	
	Potentiated inhibitory effect (lowered IC_{50} values)	No effect (No change in IC_{50} values)
OATP1B1	Asunaprevir CsA (and AM1) Dasatinib (weak) Gemfibrozil (weak) Rifampin Ritonavir (weak) Simeprevir	Saquinavir Rifamycin SV Sidenafil Clarithromycin Erythromycin Telmisartan Glibenclamide Ketoconazole
OATP1B3	Asunaprevir CsA (and AM1) Dasatinib (weak) Rifampin Simeprevir	
OAT1	(Chrysophanol) (Physcion)	Probenecid (Rhein) (Emodin) (Aloe-emodin)
OAT3	(Emodin) (Aloe-emodin) (Chrysophanol) (Physicon)	Probenecid (Rhein)

- In addition to CsA, some compounds (eg, anti-HCV drugs) showed time-dependent inhibition for OATP1B1.
- Time-dependent inhibition is reported for OATP1B3, OAT1 and OAT3.

Conclusions

The present study focused on substrate- and time-dependent inhibition of OATP1B1 to establish optimal in vitro inhibition assay conditions.

■ Substrate-dependent inhibition of OATP1B1

- >10-fold substrate-dependent K_i variability observed in some inhibitors.
- E_2G and drug probes (eg, statins) offered lower K_i values.

■ Impact of substrate-dependent K_i variation on DDI risk assessment

- CsA, RIF and GEM were judged as potential OATP1B1 inhibitors, which was consistent with clinical findings.
- However, the DDI risk of GEM (a moderate-to-weak inhibitor) could be underestimated due to potential K_i variability. In OATP1B1 inhibition assay, E_2G or drug probes will be useful to avoid false-negative DDI prediction potentially caused by substrate-dependent K_i variability.

■ Fluorescent substrate for OATP1B1

- Fluorescence-based inhibition assay with a new fluorescence probe substrate DCF will be useful particularly at the early stage of drug development.

■ Time-dependent inhibition of OATP1B1 by CsA

- Inhibitory effect of CsA on OATP1B1 was 3- to 5-fold potentiated by CsA pre-incubation, and the K_i values obtained after CsA pre-incubation was similar to in vivo K_i values estimated by PBPK modeling.
- In the future, we may need to address time-dependent inhibition for other transporters.