Extrapolation of In Vivo Hepatic Clearance from *In Vitro* Uptake Clearance by Suspended Human Hepatocytes (IVIVE) for Anionic Drugs with High Binding to Human Albumin: Improvement of IVIVE by Considering the "Albumin-Mediated" Hepatic Uptake Mechanism Based on the Facilitated-Dissociation Model

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The material includes 2 supplementary Figures, and 3 supplemental Tables.

Supplemental Figure 1. Time profiles of the uptake of OATP substrates in suspended human hepatocytes with various concentration of human serum albumin (HSA).

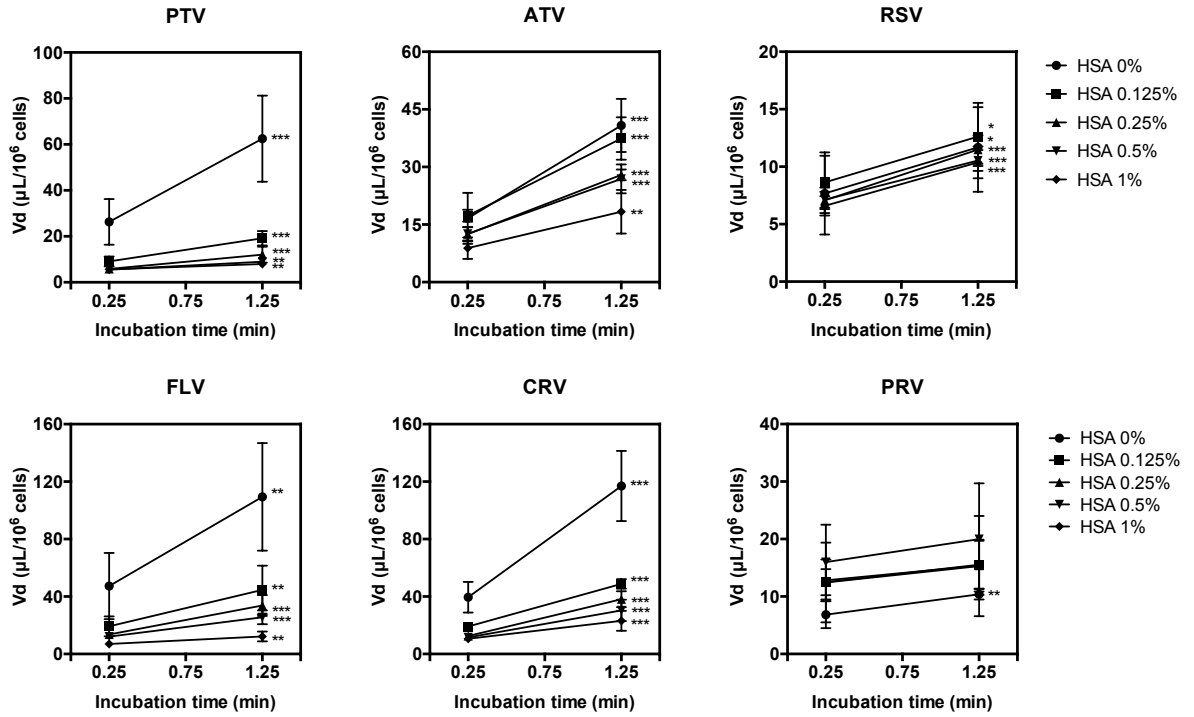
Supplemental Figure 2. The hepatic unbound uptake clearance in the presence of the albumin ($PS_{u,inf}(+)$) of clinical OATPs substrates in suspended human hepatocytes.

Supplemental Table 1. LC–MS/MS conditions for eleven drugs

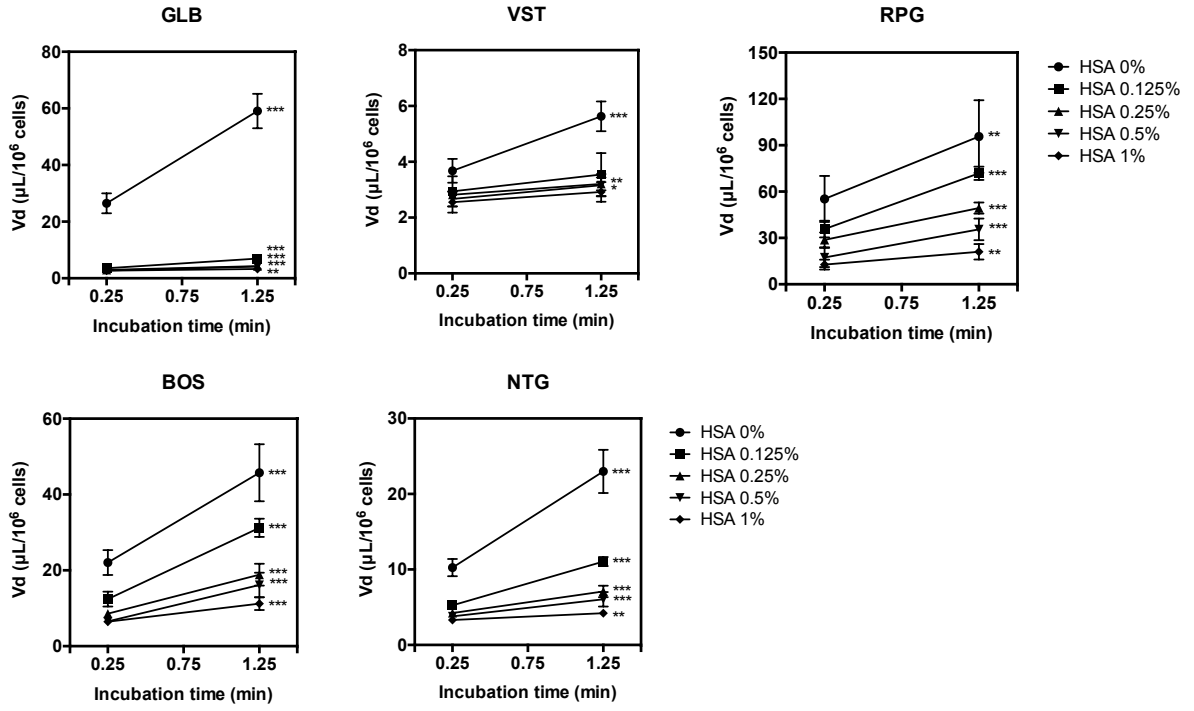
Supplemental Table 2. The reported values of K_m and the uptake clearance of eleven drugs in suspended hepatocytes

Supplemental Table 3. Sources of *in vitro* and *in vivo* parameters

(A) Cassette A

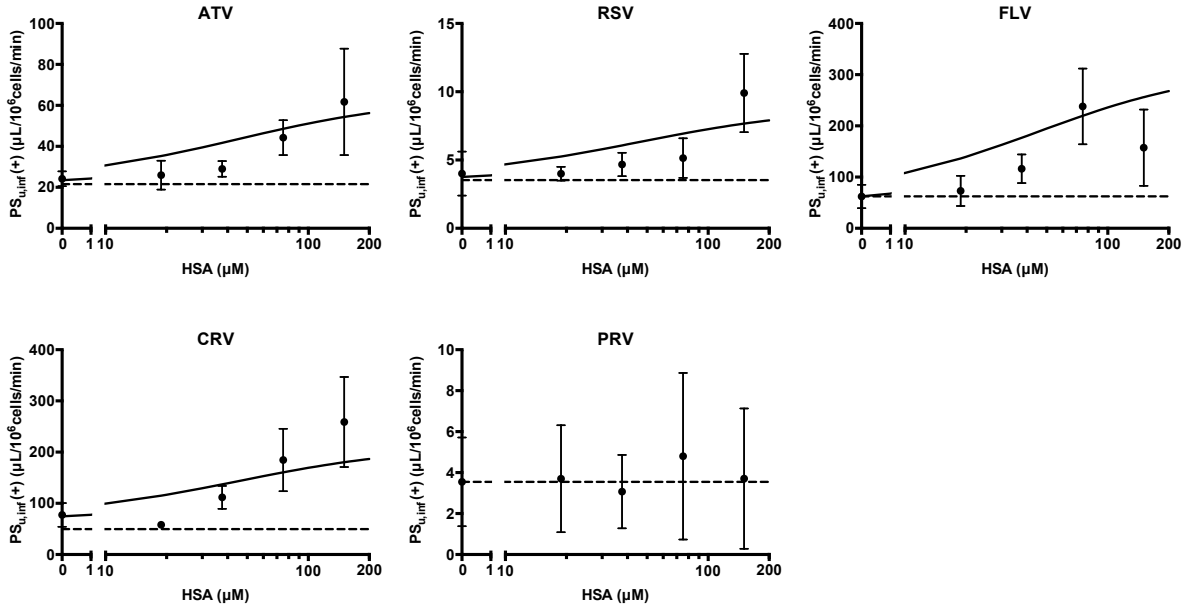


(B) Cassette B

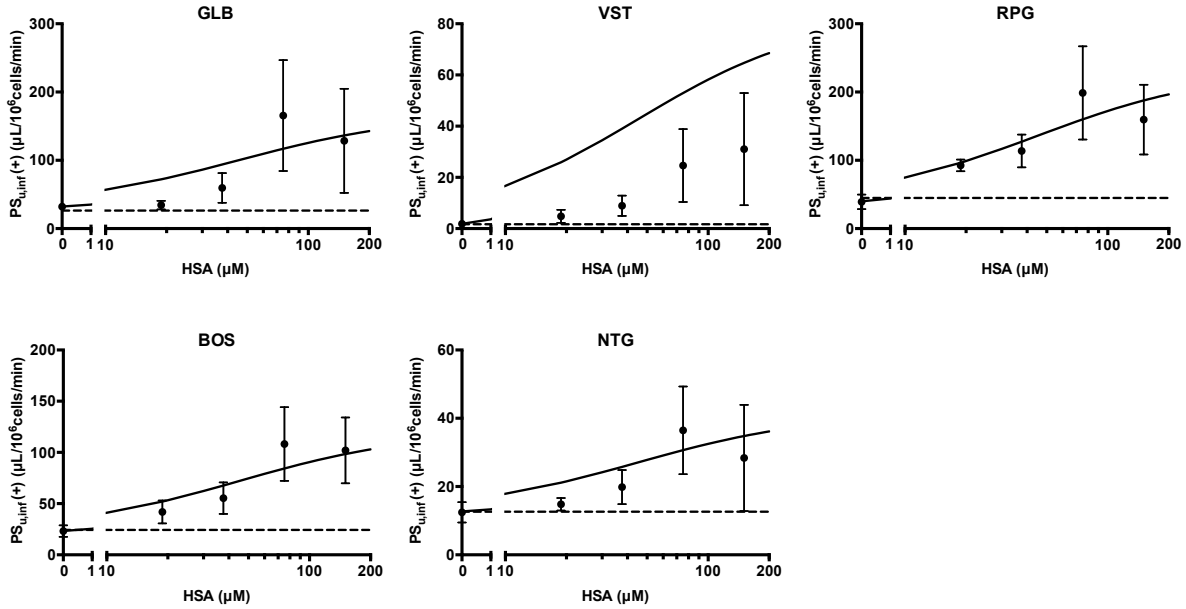


Supplemental Figure 1. Time profiles of the uptake of OATP substrates in suspended human hepatocytes with various concentration of human serum albumin (HSA) (mean ± SD, n=8). Panel (A) and (B) is cassette A and B set, respectively. PTV, pitavastatin; ATV, atorvastatin; RSV, rosuvastatin; FLV, fluvastatin; CRV, cerivastatin; PRV, pravastatin; GLB, glibenclamide; VST, valsartan; RPG, repaglinide; BOS, bosentan; NTG, nateglinide. *$P < 0.05$ vs @0.25 min, ** $P < 0.005$ vs @0.25 min, ***$P < 0.0005$ vs @0.25 min

(A) Cassette A



(B) Cassette B



Supplemental Figure 2. The hepatic unbound uptake clearance in the presence of the albumin ($PS_{\mathrm{u,inf}}(+)$) of clinical OATPs substrates in suspended human hepatocytes. The filled circle, solid line and broken line represent the calculated $PS_{\mathrm{u,inf}}(+)$ (mean ± SD, n=8), the fitted line by Tsao's model (Eq. 8), and the theoretical line based on free drug theory, respectively. Panel (A) and (B) is cassette A and B set, respectively. PTV, pitavastatin; ATV, atorvastatin; RSV, rosuvastatin; FLV, fluvastatin; CRV, cerivastatin; PRV, pravastatin; GLB, glibenclamide; VST, valsartan; RPG, repaglinide; BOS, bosentan; NTG, nateglinide.

Supplemental Table 1. LC–MS/MS conditions for eleven drugs

LC–MS/MS	Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan)		
Column	Kintex C18 column (2.1 × 100 mm, 2.6 μm; Phenomenex, Torrance, CA)		
Flow rate	0.3 mL/min		
Mobile phase	A: 01% Formic acid, B: Acetonitrile		
Drugs	ESI mode	*m/z*	Gradient condition (B concentration %)
Pitavastatin	Positive	422/290	0.5min, 5% → 3.5min, 80% → 4.5min, 80% → 4.6min, 5% → 6min, 5%
Atorvastatin	Positive	559/440	
Rosuvastatin	Positive	482/258	
Fluvastatin	Negative	410/215	
Cerivastatin	Positive	460/356	
Pravastatin	Negative	423/303	
Glibenclamide	Positive	494/369	0.5min, 5% → 5.5min, 80% → 6.5min, 80% → 6.6min, 5% → 8min, 5%
Valsartan	Positive	436/291	
Repaglinide	Positive	453/230	
Bosentan	Positive	553/202	
Nateglinide	Negative	317/113	

Supplemental Table 2. The reported values of K_m and the uptake clearance of eleven drugs in suspended hepatocytes

Drugs	K_m (μM)		Uptake clearance without HSA (uL/min/10^6cells, $PS_{u,inf}$)		
	Values	References	Values	References	Our study
Pitavastatin	2.99, 1.59	[1],[2]	26.7, 40.1	[1],[2]	36.2
Atorvastatin	0.18	[3]	211	[3]	24.2
Rosuvastatin	10.3, 11, 4	[2],[4],[5]	11.1, 4.1, 1.2	[2],[4],[5]	4.0
Fluvastatin	(4.8, 12)*	[6],[7]	-		62.1
Cerivastatin	18.3	[8]	284	[8]	77.5
Pravastatin	2.25, 76.7, 11.5	[2],[9],[10]	2.8, 1.8, 0.9,	[2],[9],[10]	3.6
Glibenclamide	(1.24, 2.0)*	[6],[11]	-		32.2
Valsartan	10.4	[2]	2.9	[2]	1.9
Repaglinide	12.8	[2]	52.3, 41.0	[2],[3]	39.2
Bosentan	1.3, 22.5	[2].[3]	36.1, 17.9	[2].[3]	23.2
Nateglinide	(36.4)*	[6]	-		12.5

* OATP1B1 over-expressed HEK cells

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Supplemental Table 3. Sources of *in vitro* and *in vivo* parameters

Drugs	R_B	Clinical data after a single intravenous dose					References
		$CL_{t,p}$	$CL_{r,p}$	$CL_{t,B}$	$CL_{r,B}$	$CL_{h,B}$	
		(mL/min/kg)					
Pitavastatin	0.58	5.63	0.04	9.71	0.0679	9.64	[1],[2]
Atorvastatin	0.66	8.93	Negligible	13.5	Negligible	13.5	[1],[2],[3]
Rosuvastatin	0.69	11.60	3.24	16.8	4.70	12.1	[4],[5]
Fluvastatin	0.62	8.70	Negligible	14.0	Negligible	14.0	[6],[7]
Cerivastatin	0.58	2.90	Negligible	5.00	Negligible	5.00	[8],[9]
Pravastatin	0.56	13.50	6.30	24.1	11.3	12.9	[1],[10]
Glibenclamide	0.46	1.06	Negligible	2.30	Negligible	2.30	[11],[12]
Valsartan	0.55	0.52	0.15	0.947	0.269	0.678	[13],[14]
Repaglinide	0.62	7.80	0.62	12.6	1.00	11.6	[15],[16]
Bosentan	0.83	2.39	0.02	2.88	0.0259	2.85	[2],[17]
Nateglinide	0.5	1.53	Negligible	3.05	Negligible	3.05	[18]

R_B, the blood partitioning; $CL_{t,p}$ total plasma clearance; $CL_{r,p}$, renal plasma clearance; $CL_{t,B}$, total blood clearance ($CL_{t,p}/R_B$); $CL_{r,B}$, renal blood clearance ($CL_{r,p}/R_B$); $CL_{h,B}$, hepatic blood clearance ($CL_{t,B} - CL_{r,B}$).

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