

PAH is the only OAT1-specific substrate in the present study, and thus, further studies are necessary to elucidate the predictability of the tubular secretion of OAT1 substrates using kidney slices and to examine whether the scaling factor of 10 can be applied to OAT1- and OCT2 substrates.

The variations in the uptake activity in the major elimination organs greatly affect the systemic exposure (Kusuhara and Sugiyama, 2009). Inhibition of uptake has been reported as the underlying mechanism of drug-drug interactions (DDI) in the renal elimination (Tahara et al., 2006a; Tahara et al., 2006b; Nozaki et al., 2007b; Matsushima et al., 2009). In the present study, we investigated the mechanism of the DDI in the kidney between pravastatin and gemfibrozil using human kidney slices. The contribution of OAT-mediated uptake was examined using PAH and benzylpenicillin as inhibitors of OAT1 and OAT3. Benzylpenicillin was a more potent inhibitor of pravastatin uptake by human kidney slices than PAH (Fig. 3), suggesting that OAT3 is the main transporter (Nozaki et al., 2007a). This is consistent with a previous report which shows that OAT3, but not OAT1, can accept pravastatin as a substrate (Nakagomi-Hagihara et al., 2007). Consistent with a previous report, not only gemfibrozil but also its metabolites, gemfibrozil-M3 and gemfibrozil-glucuronide, could inhibit the uptake of pravastatin by human kidney slices with K_i values similar to those for OAT3 (3.4 μM , 2.7 μM , and 9.9 μM) (Nakagomi-Hagihara et al., 2007) (Table 3). The unbound plasma maximum concentrations ($C_{\text{max,u}}$) of gemfibrozil and its glucuronide at clinical dosages (600 mg twice a day) (Okerholm et al., 1976; Backman et al., 2002; Nakagomi-Hagihara et al., 2007) were enough to cause significant inhibition of pravastatin uptake (R values were 0.60-0.69 and 0.8, respectively), whereas that of gemfibrozil-M3 was not high enough (the R value was 0.93). Therefore, inhibition of OAT3 by gemfibrozil and gemfibrozil glucuronide could account for the DDI. It is worth mentioning that the inhibition by gemfibrozil glucuronide might appear to be biphasic (Figure 4C) although such biphasic inhibition was not observed in OAT3-transfected cells (Nakagomi-Hagihara et al., 2007). Further studies are necessary to elucidate the mechanism

underlying this discrepancy. Today, there is a growing interest in the safety of metabolites. The FDA has issued Human Metabolites in Safety Testing (MIST), guidance to assure the safety of major drug metabolites. Caution must be paid to the incidence of DDI caused by drug metabolites.

Taken together, freshly prepared kidney slices serve to predict the renal clearance, and DDI quantitatively. Figure 5 shows the reliability of the prediction of hepatic clearance of anionic drugs using cryopreserved human hepatocytes where the extrapolated hepatic uptake clearance of most of anionic drugs was comparable with the overall hepatic intrinsic clearance. Rosuvastatin as well as fluvastatin was an outlier of the correlation (Figure 5), and this holds true in rats by unknown reason (Watanabe et al., 2009b). As reported previously for fluvastatin (Watanabe et al., 2010), rat scaling factor may be able to improve the prediction. Unfortunately, because the hepatic elimination of rosuvastatin is blood-flow limited, the scaling factor could not be precisely obtained by rat studies. Taken together, *in vitro* uptake studies using human hepatocytes often lead to accurate prediction, however, it is recommended to perform IVIVE in animal study to obtain highly reliable prediction. *In vitro* transport experiments would be able to predict the elimination pathway of anionic drugs from the systemic circulation in early stage of drug development.

In conclusion, the present study suggests that hepatic and renal clearances of anionic drugs are predictable based on *in vitro* uptake studies using human cryopreserved hepatocytes and kidney slices. Moreover, kidney slices can be used for predicting clinically relevant DDI caused by alterations in renal uptake activity. These *in vitro* experimental systems will be applicable to drug discovery and development studies for the prediction of organ clearances in the liver and kidney.

Authorship Contributions

Participated in research design: Ta Watanabe, To Watanabe, Kusuhara, Maeda and Sugiyama.

Conducted experiments: Ta Watanabe, To Watanabe, Debori, kondo, Nakayama and Horita.

Contributed new reagents or analytic tools: Ogilvie, Parkinson and Hu.

Performed data analysis: Ta Watanabe, To Watanabe and Debori.

Wrote or contributed to the writing of the manuscript: Ta Watanabe, Kusuhara, Ogilvie, Parkinson and Sugiyama.

Other: Kusuhara and Sugiyama acquired funding for the research.

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Legends for Figures

Fig. 1. Time profiles of the uptake of [³H]-rosuvastatin, [³H]-pravastatin, [³H]-pitavastatin, [³H]-valsartan, and [³H]-olmesartan by human kidney slices. The uptake of these drugs was determined at two concentrations at 37°C. Final concentrations of the drugs were as follows: 0.1 μM and 100 μM for rosuvastatin, pitavastatin and valsartan; 0.1 μM and 500 μM for pravastatin; 0.01 μM and 100 μM for olmesartan. Each point represents the mean ± SE (n = 3 slices from one kidney).

Fig. 2. Comparison between the uptake clearance by human kidney slices ($CL_{\text{uptake,slice}}$) and the observed renal secretion intrinsic clearance ($CL_{\text{sec,int,all}}$) (Panel A) and between the predicted renal secretion clearance ($CL_{\text{sec,predicted}}$) and the observed one (CL_{sec}) (Panel B). $CL_{\text{sec,int,all}}$ was calculated based on Eqs. 1 to 4. $CL_{\text{sec,predicted}}$ was estimated from Eqs. 1 to 4, assuming that $CL_{\text{uptake,slice}}$ was equal to the renal overall intrinsic clearance. Plots represent the following: 1, rosuvastatin; 2, pravastatin; 3, pitavastatin; 4, valsartan; 5, olmesartan; 6, Trichlormethiazide; 7, PAH; 8, fexofenadine; 9, methotrexate; and 10, benzylpenicillin. The solid line and the dashed lines represent the line of unity and the lines of the 1:3 and 3:1 correlations, respectively. Circles and diamonds indicate drugs taken up into the kidney by mainly OAT3 and OAT1, respectively. Triangles show drugs, the dominant transporter of which is unclear.

Fig. 3. Inhibitory effect of PAH and benzylpenicillin on the uptake of pravastatin by human kidney slices. The uptake of [³H]-pravastatin (0.1 μM) was determined in the presence and absence of unlabeled PAH (open columns) and benzylpenicillin (closed columns), and in the presence of excess unlabeled pravastatin (500 μM) for 15 min at 37°C. The values are shown as a percentage of the uptake in the absence of inhibitors and an excess of pravastatin. Each value represents the mean ± SE (n = 6 slices). The absolute uptake clearance of

pravastatin at 0.1 μM in the absence of inhibitors was 2.86 ± 0.11 ml/g kidney/15 min (mean \pm SE).

Fig. 4. Inhibitory effect of gemfibrozil and its metabolites, gemfibrozil-M3 and gemfibrozil-glucuronide on the uptake of pravastatin by human kidney slices. The uptake of [^3H]-pravastatin (0.1 μM) was determined in the presence and absence of unlabeled gemfibrozil (A), gemfibrozil-M3 (B) and gemfibrozil-glucuronide (C) for 15 min at 37°C. The values are shown as a percentage of the uptake in the absence of inhibitors. Each value represents the mean \pm SE (n = 6 slices). The absolute uptake clearance of pravastatin at 0.1 μM in the absence of inhibitors was 2.86 ± 0.11 ml/g kidney/15 min (mean \pm SE). Dashed lines represent the ratio of the uptake of [^3H]-pravastatin determined in the presence of excess unlabeled pravastatin to that in the absence of excess pravastatin.

Fig. 5. Comparison between the uptake clearance by hepatocytes ($\text{CL}_{\text{uptake,hep}}$) and the observed hepatic overall intrinsic clearance ($\text{CL}_{\text{h,int,all}}$)

Plots represent the following: 1, rosuvastatin; 2, pravastatin; 3, valsartan; 4, olmesartan; 5, candesartan; 6, pitavastatin; 7, atorvastatin; 8, fluvastatin and 9, cerivastatin. The solid line and the dashed lines represent the line of unity and the lines of 1:3 and 3:1 correlations, respectively.

Table 1. Uptake clearance of drugs by human kidney slices

	Batch Nos.	uptake of test drug			normalized value ^a	CL _{uptake,slice} ^b
		tracer (T)	excess (E)	T-E		
		ml/g kidney/15 min				ml/min/kg
rosuvastatin	1	8.02 ± 1.55	2.69 ± 0.06	5.33 ± 1.55	11.9	3.30
pravastatin	2	2.86 ± 0.11	0.84 ± 0.05	2.02 ± 0.12	1.77 ± 0.13	0.447
	3	2.62 ± 0.31	0.76 ± 0.04	1.86 ± 0.31	1.68 ± 0.38	
	4	2.15 ± 0.10	0.71 ± 0.03	1.43 ± 0.10	1.71 ± 0.27	
	5	2.05 ± 0.40	1 ± 0.12	1.05 ± 0.42	1.29 ± 0.23	
pitavastatin	6	20.6 ± 1.36	15.3 ± 1.52	5.27 ± 2.04	2.55	0.957
	8	15.3 ± 2.34	11.8 ± 1.04	3.50 ± 2.56	4.36 ± 0.24	
valsartan	1	8.28 ± 1.06	2.34 ± 0.34	5.94 ± 1.11	13.3	2.38
	7	6.38 ± 1.88	3.34 ± 0.12	3.04 ± 1.89	3.88 ± 0.24	
olmesartan	6	16.0 ± 0.72	1.67 ± 0.11	14.3 ± 0.73	6.93 ± 0.66	3.13
	7	13.9 ± 1.59	1.65 ± 0.04	12.2 ± 1.59	15.6 ± 0.76	
trichlormethiazide	3	5.18 ± 0.79	2.12 ± 0.06	3.06 ± 0.79	2.77 ± 0.63	1.01
	4	6.72 ± 0.86	2.94 ± 0.33	3.78 ± 0.92	4.50 ± 0.71	
PAH	2	2.24 ± 0.31	0.55 ± 0.01	1.69 ± 0.31	1.48 ± 0.11	0.482
	3	2.14 ± 0.22	0.60 ± 0.03	1.54 ± 0.22	1.40 ± 0.32	
	4	1.63 ± 0.17	0.63 ± 0.00	1.00 ± 0.17	1.19 ± 0.19	
	5	2.98 ± 0.36	0.74 ± 0.32	2.24 ± 0.49	2.75 ± 0.49	
	6	4.98 ± 0.21	1.12 ± 0.06	3.86 ± 0.22	1.87 ± 0.18	
fexofenadine	(1)	2.60 ± 0.29	0.91 ± 0.21	1.68 ± 0.36	1.25 ± 0.19	0.347
methotrexate	(2)	1.26	0.51	0.75	0.61	0.168
benzylpenicillin	1	1.56 *	0.74 *	0.82 *		0.508
	2	2.70 ± 0.15	0.61 ± 0.02	2.09 ± 0.15		
	3	2.71 ± 0.46	0.69 ± 0.07	2.02 ± 0.46		
	4	2.21 ± 0.24	0.68 ± 0.00	1.54 ± 0.24		
	5	2.00 ± 0.11	0.51 ± 0.24	1.49 ± 0.26	1.72 ^c ± 0.09	
	6	4.26 ± 0.27	0.46 ± 0.23	3.79 ± 0.36		
	7	2.17 ± 0.07	0.74 ± 0.02	1.43 ± 0.07		
	8	2.31 *	0.84 *	1.47 *		

Uptake clearance of drugs by human kidney slices was determined at 37°C at two (trace and excess) concentrations. Final concentrations of the drugs were as follows: 0.1 µM and 100 µM for rosuvastatin, pitavastatin and valsartan; 0.1 µM and 500 µM for pravastatin; 0.01 µM and 100 µM for olmesartan; 0.1 µM and 1000 µM for PAH; 1 µM and 1000 µM for benzylpenicillin; 10 µM and 1000 µM for trichlormethiazide. The uptake clearance was calculated by dividing

the uptake volume at 15 min by the drug concentration in the incubation buffer. Each value represents the mean \pm SE (n = 3 slices). Benzylpenicillin was used as the standard compound throughout the study for normalization of the uptake of the test drugs by different batch of kidney slices.

^a Uptake of test drugs was corrected by the ratio of the saturable uptake of benzylpenicillin in the same batch of human kidney to the average value (1.83)

^b Normalized uptake clearance were scaled up to the in vivo value per body weight using the following physiological scaling factors, 4.43 g kidney/kg body weight (Davies and Morris, 1993). Average values were used for the extrapolation for rosuvastatin, pravastatin, pitavastatin, valsartan, olmesartan, trichlormethiazide and PAH.

^c average value used for the normalization

* average value of two slices

⁽¹⁾ (Matsushima et al., 2009) T-E of benzylpenicillin was 2.47 ml/g kidney/15 min

⁽²⁾ (Nozaki et al., 2007b) T-E of benzylpenicillin was 2.26 ml/g kidney/15 min

Table 2. Kinetic parameters of drugs regarding hepatic and renal clearance.

	$CL_{\text{uptake, hep}}$	$CL_{\text{uptake, slice}}^a$	f_u^b	R_B^b	f_B	$CL_{h, \text{int, all}}^b$	$CL_{\text{sec, int, all}}^b$	Scaling factor ^c	CL_{sec}^b
	ml/min/kg	ml/min/kg				ml/min/kg	ml/min/kg		ml/min/kg
Rosuvastatin	15.1	3.30	0.12	0.69	0.174	104	27.0	8.2	3.92
Pravastatin	13.8 ^d	0.447	0.55	0.56	0.989	23.3	16.6	37	9.47
Pitavastatin	446 ^d	0.957	0.0052	0.58	0.009	411	3.34	3.5	0.03
Valsartan	18.9	2.38	0.04	0.55	0.073	8.95	1.80	0.7	0.129
Olmесartan	12.1	3.13	0.01	0.55	0.018	18.6	11.1	3.5	0.204
Trichlormethiazide	-	1.01	0.29	0.55	0.527	-	10.7	11	4.54
PAH	-	0.482	0.83	0.70	1.19	-	N.A.	N.A.	13.6
Fexofenadine	-	0.347 ^f	0.31	0.55	0.564	-	1.52	4.4	0.658
Metotrexate	-	0.168 ^g	0.37	0.83	0.446	-	1.40	8.3	1.13
benzylpenicillin	-	0.508	0.56	0.66	0.843	-	5.71	11	5.26
Candesartan	44.8	-	0.0027	0.55	0.005	67.2	-	-	-
Atorvastatin	169 ^d	-	0.0511	0.61	0.084	329	-	-	-
Fluvastatin	351 ^d	-	0.0037	0.52	0.007	3760	-	-	-
Cerivastatin	746 ^e	-	0.007	0.58	0.012	485	-	-	-

$CL_{\text{uptake, hep}}$, uptake clearance by human hepatocytes; $CL_{\text{uptake, slice}}$, corrected saturable uptake clearance by human kidney slices; $CL_{h, \text{int, all}}$, observed hepatic overall intrinsic clearance; $CL_{\text{sec, int, all}}$, observed renal secretion overall intrinsic clearance; CL_{sec} , observed renal secretion clearance; N.A., not applicable

^a saturable uptake clearance corrected by uptake of benzylpenicillin. The details of this estimation are described in the text and Table 1.

^b references cited are listed in the Appendix (Supplemental Table 1).

^c Scaling factors were calculated by dividing $CL_{\text{sec, int, all}}$ by $CL_{\text{uptake, slice}}$.

^d (Watanabe et al., 2010a)

^e (Shitara et al., 2003)

∫ (Matsushima et al., 2009)

⌘ (Nozaki et al., 2007b)

Table 3. Kinetic parameters of gemfibrozil-related compounds to predict the DDI between pravastatin and gemfibrozil.

	Ki	C _{max}	fu ^d	C _{max,u}	R
	μM	μM			
Gemfibrozil	1.47 ± 0.48	100-150 ^{a, b}	0.00648	0.65-0.97	0.60-0.69
Gemfibrozil-M3	3.97 ± 1.49	24 ^c	0.0123	0.30	0.93
Gemfibrozil-glucuronide	9.05 ± 8.43	20 ^b	0.115	2.3	0.80

Inhibitory effect of gemfibrozil-related compounds on the uptake of pravastatin by human kidney slices was examined. The Ki values were determined by non-linear regression analysis and are represented as the mean ± S.D.

^a (Backman et al., 2002)

^b (Okerholm et al., 1976)

^c (Nakagomi-Hagihara et al., 2007)

^d (Shitara et al., 2004)

Fig. 1

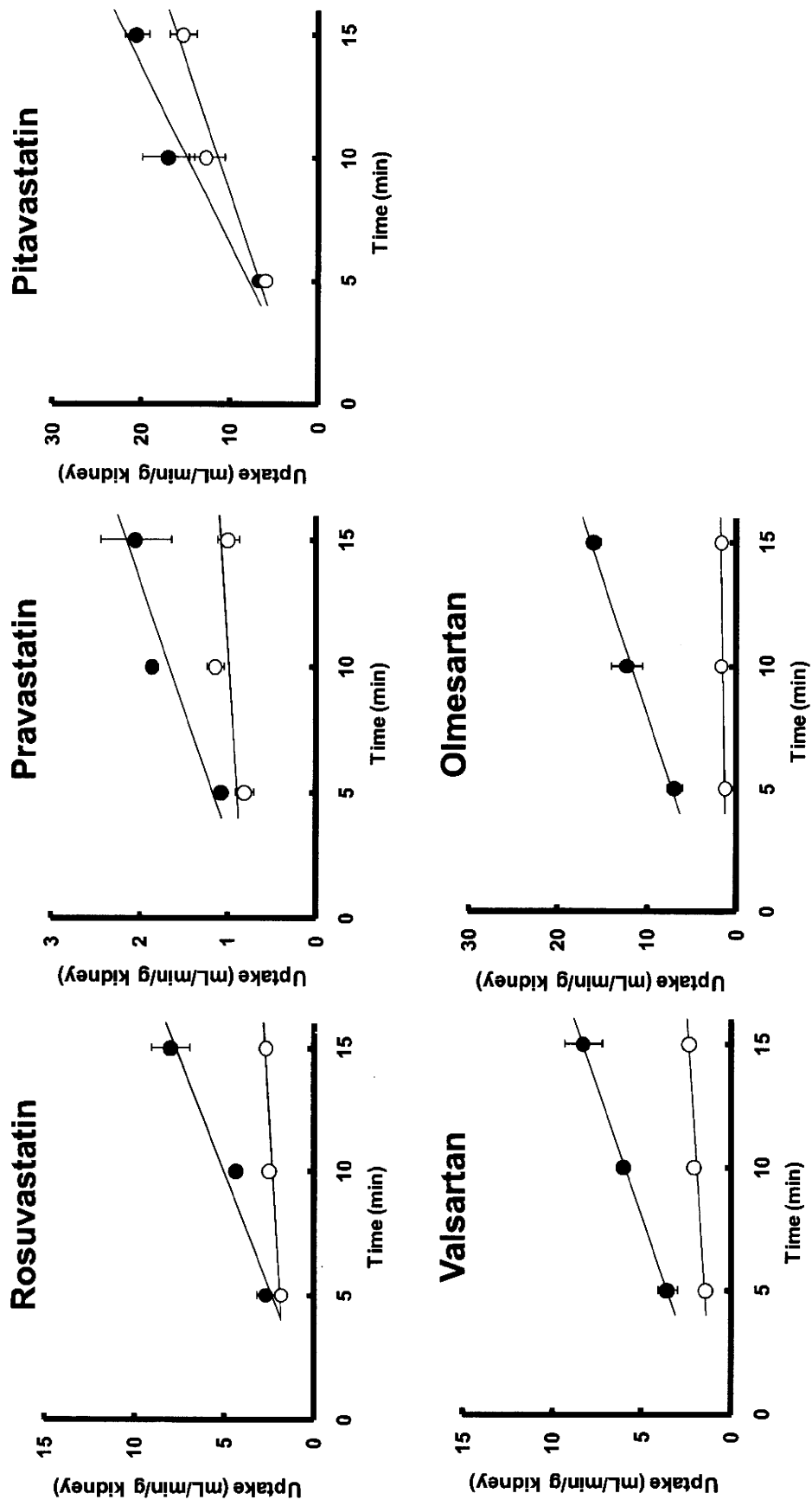


Fig. 2

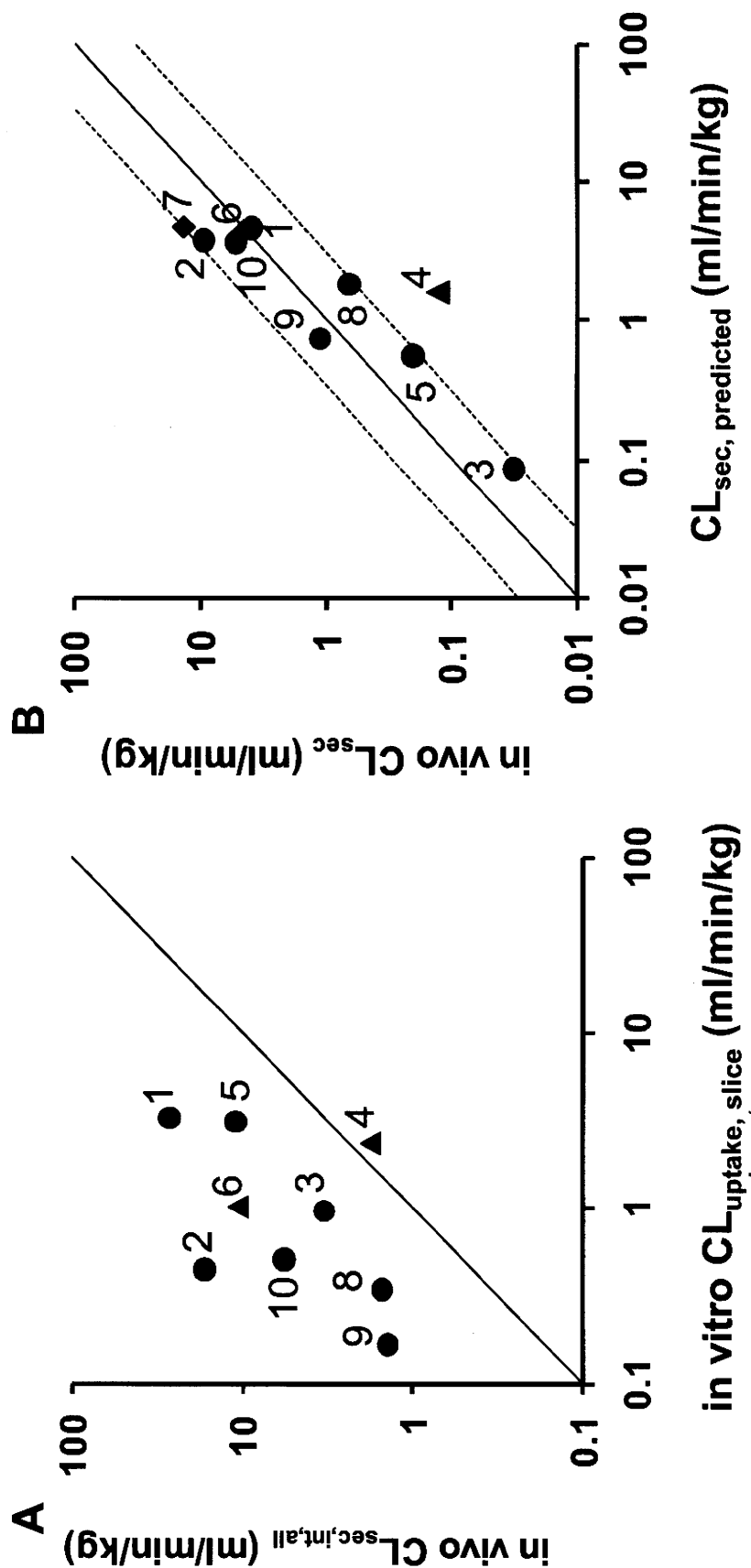


Fig. 3

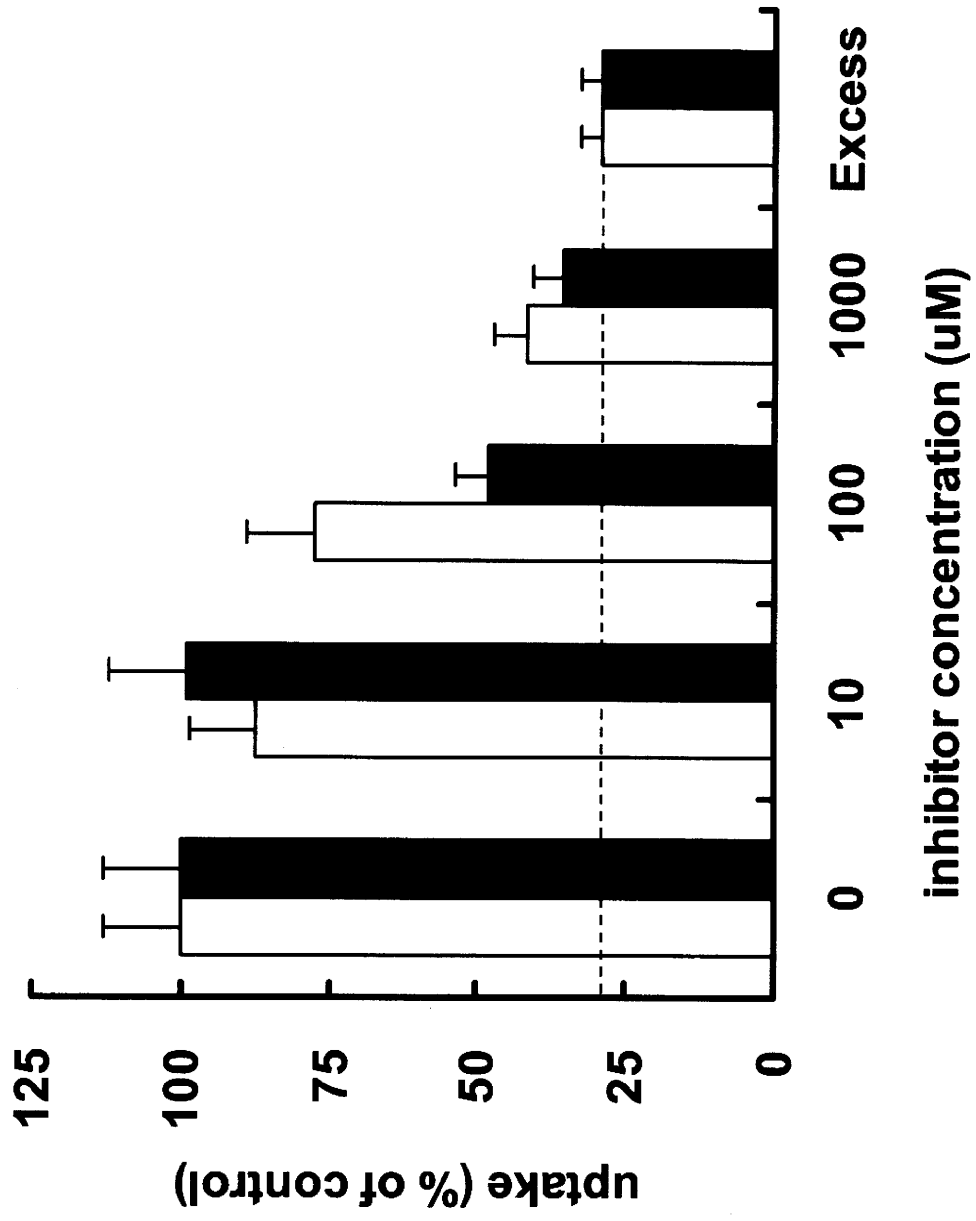


Fig. 4

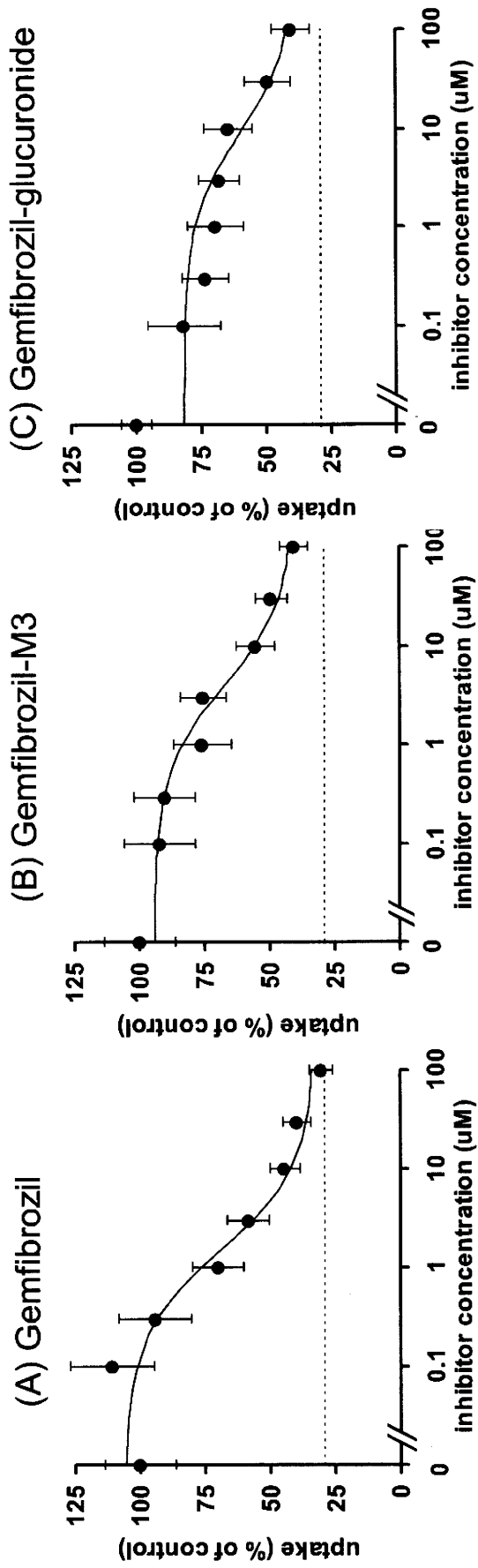


Fig. 5

