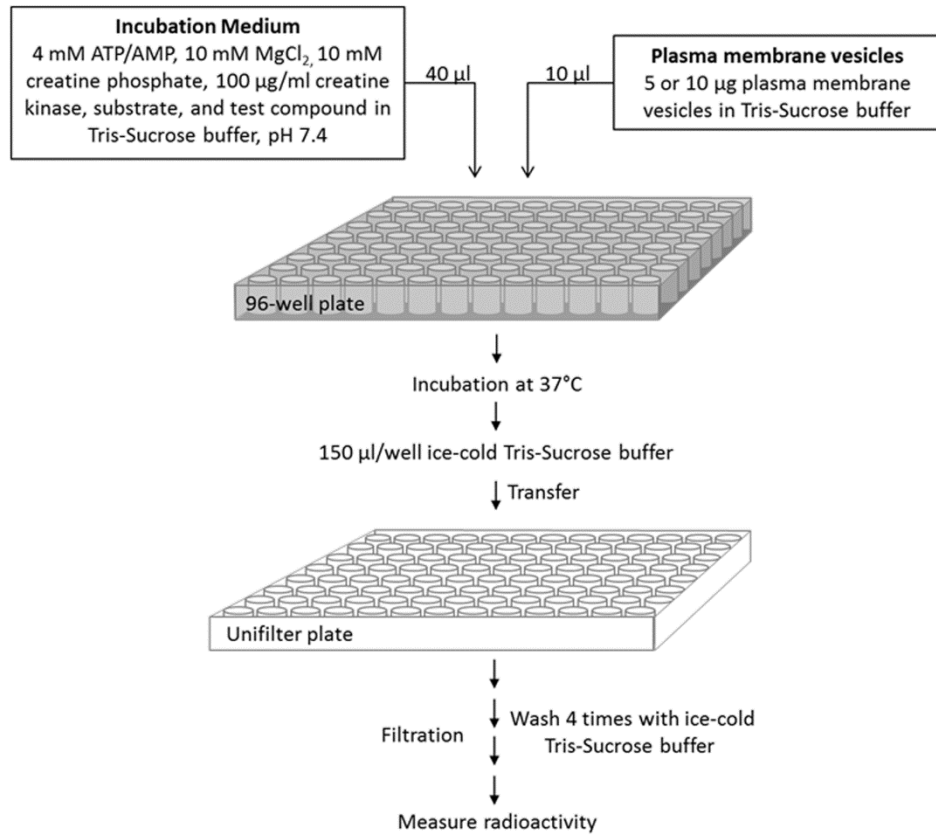


DMD #54304

**Risk Factors for Development of Cholestatic Drug-induced Liver Injury:
Inhibition of Hepatic Basolateral Bile Acid Transporters MRP3 and
MRP4**

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Drug Metabolism and Disposition



Supplemental Figure 1. Schematic of the high-throughput assay to study MRP3 and MRP4 inhibition.

Supplementary Table 1. Human protein binding, C_{max} , unbound C_{max} ($C_{max,u}$), maximum daily dose (MDD) in man, main route of excretion, extent of liver metabolism and involvement of phase II metabolism for the 88 investigated non-cholestatic and cholestatic compounds.

Drug	Protein binding (%)	C_{max} ($\mu\text{g}/\text{ml}$)	$C_{max,u}$ (μM)^a	MDD in man (mg/day)^b	Standard dose (mg/day)^c	Main Route of Excretion^d	Liver Metabolism^e	Phase II Metabolism
Non-cholestatic BSEP non-inhibitors								
5-Fluorouracil	8.0 ²	17.7 ¹	125	4420 iv ¹	2380	Urinary ¹⁹	Extensive ²⁰	No
Alprenolol	80.0 ²	0.14 ³	0.11	800 ¹	300	Urinary	Extensive	No
Antipyrine	10.0	25 ³	120	800	800	N/A	N/A	N/A
Aspirin	80.0 ¹	200 ³	222	4000 ¹	2037.5	Urinary	Extensive	No
Caffeine	36.0 ¹	17.3 ¹	57	1200 ¹	632.5	Urinary	Extensive	No
Chlorpheniramine	70.0 ²	0.08 ²	0.083	24 ¹	24	Urinary	Extensive	No
Clopamide	46.0 ¹	0.18 ²¹	0.27	40 ¹	22.5	Urinary	N/A	No
Dexamethasone	77.0 ¹⁸	0.10 ²²	0.075	24 ¹	4.88	Urinary	Extensive ²³	Yes ²⁴
Diphenhydramine	76.0 ¹	0.23	0.22	300 ¹	200	Urinary	Extensive	Yes
Doxorubicin	74.0 ¹	0.95	0.45	75/ m ² /3 week iv ¹	81	Biliary	Extensive	No

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Etoposide	96.0 ²	4.7	0.32	2 g/m ² / 24 h iv ¹	85	Urinary	Extensive	No
Fluorescein	85.0 ¹	35 ¹	15.8	500 iv ¹	500	Urinary	Extensive	Yes
Metoclopramide	30.0 ¹	0.1 ¹	0.23	60 ⁶	370	Urinary	Non-significant	Yes
Nadolol	28.0 ¹	0.25 ³	0.58	320 ¹	45	Urinary	Non-significant	No
Naloxone	45.9 ²⁵	0.03 ³	0.050	3 ¹	1.2	Urinary	Extensive	Yes
Phenformin	12.0 ¹	0.15 ¹	0.65	2550 ¹	81.25	Urinary	Non-significant	No
Probenecid	80.0 ¹	150 ³	100	2000 ¹	1125	Urinary	Extensive	Yes
Quinidine	80.0 ¹	8 ¹	4.9	1600 ⁶	1200	Urinary	Extensive	No
Tacrine	55.0 ¹	0.016 ⁵	0.036	160 ¹	40	Urinary ²⁶	Extensive	No
Terbutaline	20 ²⁷	0.03 ³	0.11	15 ¹	11.25	Urinary	Non-significant	Yes
Theophylline	40.0 ¹	15 ¹	50	900 ¹	450	Urinary	Extensive	No
Timolol	10.0 ¹	0.05 ³	0.14	60 ¹	35	Urinary	Extensive	No
Triamterene	55.0 ¹	0.1 ³	0.18	300 ¹	75	Urinary	Extensive	Yes
Vinblastine	98.0 ¹	0.001 ²⁸	0.00002	11.1/m ² iv ¹	12.6	Biliary	Extensive	No

Cholestatic BSEP non-inhibitors

Bezafibrate	95.0 ¹	14.3 ¹	2.0	600 ¹	500	Urinary	N/A	Yes
Carbamazepine	74.0 ²	12 ³	13	1600 ¹	1000	Urinary	Extensive	No

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Chloramphenicol	50.0 ¹	25 ³	39	7000 ¹	5250	Urinary	Extensive	No
Chlorpromazine	90.0 ¹	0.3 ¹	0.094	2000 ¹	400	Urinary (P.I.)	Extensive	No
Chlorpropamide	60.0 ¹	150 ³	217	750 ¹	300	Urinary	Extensive	No
Cimetidine	13.0 ¹	3.9 ¹	13	1600 ¹	800	Urinary	Extensive	Yes
Desipramine	73.0 ¹	0.5 ³	0.45	300 ¹	150	Urinary	Extensive	Yes
D-Penicillamine	80.0 ⁶	5.6 ³	7.5	4000 ¹	187.5	Urinary (P.I.)	Extensive	No
Famotidine	10.0 ¹	104 ¹	277	640 ¹	360	Urinary	Non-significant	Yes
Fluoxetine	94.0 ²	0.477 ¹	0.09	80 ¹	50	Urinary	Extensive	No
Furosemide	91.0 ¹	6 ³	1.6	600 ¹	90	Urinary	Non-significant	No
Haloperidol	90.0 ¹	0.02 ²	0.006	15 ¹	1.25	Urinary	N/A	Yes
Ibuprofen	99.0 ²	57 ¹	2.8	3200 ¹	1000	Urinary	Extensive	Yes
Maprotiline	88.0 ¹	0.25 ³	0.11	225 ¹	112.5	Urinary	N/A	No
Metformin	0.0 ²	1 ³	8	2550 ¹	1250	Urinary	Non-significant	No
Nitrofurantoin	62.0 ²	5 ³	8.0	400 ¹	300	Urinary (P.I.)	N/A	No
Nortriptyline	86.0 ¹	0.2 ³	0.11	100 ¹	87.5	Urinary	Extensive	No
Promethazine	93.0 ¹	0.4 ³	0.10	150 ¹	37.5	Urinary	Extensive	No
Quinine	69.0 ¹	10 ²	10	2600 ¹	1800	Urinary	Extensive	Yes

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Ranitidine	15.0 ²	1 ³	2.7	300 ¹	150	Urinary	Extensive	No
Sulfasalazine	99.3 ¹	30 ³	0.53	4000 ¹	2000	Urinary	N/A	No
Sulindac	93.0 ¹	6 ³	1.2	400 ¹	350	Urinary	Extensive	Yes
Tamoxifen	98.0 ²	0.12 ²	0.006	40 ¹	30	Biliary	Extensive	No
Tolbutamide	80.0 ¹	100 ³	74	3000 ¹	1125	Urinary	Extensive	No
Trimethoprim	37.0 ²	2.5 ²	5.4	1050 ¹	150	Urinary	Non-significant	No
Verapamil	90.0 ²	0.27 ²	0.06	480 ¹	280	Urinary	Extensive	No

Non-cholestatic BSEP-inhibitors

Alpidem	99.1 ⁴	0.27 ⁴	0.006	150	150	Biliary ⁸	Extensive ⁸	Yes
Benzbromarone	99.0 ⁹	3.35 ¹⁰	0.079	200 ¹	125	Biliary ¹¹	Extensive	Yes
Buspirone	86.0 ¹	0.004	0.002	90 ¹	25	Urinary	Extensive	No
Clobetasol propionate	97.5 ⁴	0.02 ⁴	0.001	50 g/week topical ¹		Urinary	N/A	N/A
Finasteride	90.0 ²	0.37 ²	0.099	5 ¹	3	Biliary ¹²	Extensive	No
Flupirtine	80.0 ¹³	1.5 ³	0.99	600 ¹	450	Urinary ¹⁴	N/A	Yes ¹⁵
Glafenine	96.7 ⁴	0.7 ⁵	0.062	1200 ⁴	800	Urinary ¹⁶	Extensive ¹⁶	Yes ¹⁷
Lopinavir	98.0 ⁶	11.8 ¹	0.38	800 ⁶	800	Biliary	Extensive	No
Mibefradil	99.5 ¹	0.3 ¹	0.003	100 ¹	50	Biliary	Extensive	No

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Oxybutynin	99.0 ¹	0.001 ¹	0.00028	30 ¹	7.5	Biliary ¹¹	Extensive	No
Praziquantel	80.0 ²	2.2 ¹	1.4	5250 ¹	5250	Urinary	Extensive	No
Primaquine	77.0 ¹⁸	0.2 ³	0.18	30 ¹	30	Biliary ¹¹	Extensive	N/A
Sorafenib	99.5 ¹	9.9 ¹	0.106	800 ¹	800	Biliary	Extensive	Yes
Taxol	88.0 ²	1 ³	0.14	250/m ² /3 week iv ⁶	217	Biliary	Extensive	No
Tolcapone	99.9 ²	13 ³	0.048	1200 ¹	450	Urinary	Extensive	Yes
Valinomycin	N/A	N/A		N/A	N/A	N/A	N/A	N/A

Cholestatic BSEP-inhibitors

Acitretin	99.0 ¹	0.42 ¹	0.013	75 ¹	37.5	Urinary/biliary	Extensive	Yes
Clozapine	95.0 ²	0.8 ³	0.12	900 ¹	375	Urinary	Extensive	No
Cyclosporin A	90.0	0.4 ³	0.03	700 ¹	525	Biliary	Extensive	No
Dicloxacillin	88.0 ¹	91 ²	23	2000 ¹	1750	Urinary	Non-significant	No
Erythromycin Estolate	95.0 ⁴	3.08 ⁵	0.15	1000 ⁴	1000	Biliary	Non-significant	No
Fenofibrate	99.0 ¹	30 ¹	0.83	160 ¹	100	Urinary	N/A	Yes
Fluvastatin	98.0 ¹	0.42 ¹	0.02	80 ¹	50	Biliary	Extensive	Yes
Glyburide	99.0 ¹	0.2 ¹	0.004	20 ¹	10.6	Urinary/biliary	Extensive	No
Indinavir	60.0 ¹	0.013 ¹	0.008	2400 ¹	2100	Biliary	Extensive	Yes

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Indomethacin	90.0 ²	3 ³	0.84	200 ¹	125	Urinary	Extensive	No
Nifedipine	90.0 ¹	0.1 ³	0.029	180 ⁶	45	Urinary	Extensive	No
Nitrendipine	97.0 ¹	0.05 ³	0.004	40 ¹	25	Urinary	Extensive	Yes
19-Norethindrone	61.0 ¹	0.01 ⁵	0.013	5 ⁶	2.7	Urinary	Extensive	Yes
Omeprazole	95.0 ²	4 ³	0.58	360 ¹	30	Urinary	Extensive	No
Pioglitazone	99.0 ¹	1.5 ²	0.042	45 ¹	22.5	Biliary (P.I.)	Extensive	No
Rifampicin	60.0 ¹	10 ³	4.9	600 ¹	450	Biliary	Extensive	No
Rifamycin SV	80.0 ⁴	11 ⁴	3.2	600 ⁴	450	Biliary	Extensive	No
Ritonavir	98.0 ¹	11.2 ¹	0.31	1200 ¹	600	Biliary	Extensive	No
Rosiglitazone	99.8 ¹	0.275 ²	0.0015	8 ¹	4	Urinary	Extensive	Yes
Simvastatin	94.0 ²	0.06 ⁷	0.008	80 ¹	22.5	Biliary	Extensive	No
Ticlopidine	98.0 ²	2.1 ⁵	0.14	500 ¹	500	Urinary	Extensive	No
Troglitazone	99.0 ¹	2.8 ⁵	0.064	600 ¹	400	Biliary	Extensive	Yes

^a Calculated from C_{max} and protein binding

^b Administered orally if not stated otherwise.

^c If a dose range was recommended (e.g. for different indications), the average of minimum and maximum recommended daily dose was calculated. If dosing was given as mg/kg or mg/m², the maximum and average dose was calculated based on an adult with 70 kg and a body surface area of 1.7.

^d Compounds were categorized as urinary vs. biliary if >50% of the reported elimination occurred by a given pathway.

^e Compounds with $\geq 50\%$ of hepatic metabolism were categorized as “extensive”; compounds $< 50\%$ were categorized into the “non-significant” group.

N/A, Not Available, P.I., packaging insert

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Supplemental Table 2. Relationship between the type of liver injury and the extent of metabolism. Compounds with $\geq 50\%$ hepatic metabolism were categorized as extensive, whereas compounds with $< 50\%$ hepatic metabolism or no hepatic metabolism were categorized as not extensive. Compounds with no information on the extent of hepatic metabolism (n=11) were excluded from the analysis. Odds ratio estimates are shown with their 95% CIs.

All compounds (n=77)			
	Cholestatic	Non-cholestatic	Odds Ratio
Extensive	36	31	0.77 [0.15, 3.62]
Not extensive	6	4	
BSEP non-inhibitors (n=43)			
	Cholestatic	Non-cholestatic	Odds Ratio
Extensive	17	18	0.94 [0.15, 5.96]
Not extensive	4	4	
BSEP inhibitors (n=34)			
	Cholestatic	Non-cholestatic	Odds Ratio
Extensive	19	13	0.00 [0.00, 5.61]
Not extensive	2	0	

Supplementary Table 3. Relationship between the type of liver injury and the primary route of excretion. Compounds were categorized as urinary or biliary if >50% of the reported elimination occurred by a given pathway. Four compounds were excluded because the route of excretion was not available, or the same extent of biliary and urinary excretion was reported. Odds ratios with 95% confidence intervals are shown.

All compounds (n=84)			
	Cholestatic	Non cholestatic	Odds Ratio
Biliary excretion	11	11	0.74 [0.25, 2.22]
Urinary excretion	35	26	
BSEP non-inhibitors (n=49)			
	Cholestatic	Non cholestatic	Odds Ratio
Biliary excretion	1	2	0.42 [0.01, 8.75]
Urinary excretion	25	21	
BSEP inhibitors (n=35)			
	Cholestatic	Non cholestatic	Odds Ratio
Biliary excretion	10	9	0.67 [0.14, 3.13]
Urinary excretion	10	6	

Supplementary Table 4. Association between maximal unbound concentration $C_{\max,u}$, maximal daily dose (MDD), average daily dose (Avg. dose), MRP4 and BSEP inhibition for non-cholestatic and cholestatic drugs. Valinomycin was excluded from the analysis due to missing information on C_{\max} and dose. Data are presented as median values.

TYPE OF LIVER INJURY			
All compounds (n=87)			
	$C_{\max,u}$ (μM)	MDD (mg/day)	Avg. dose (mg/day)
Non-cholestatic (n = 39)	0.27	310	132.5
Cholestatic (n = 48)	2.05	490	300
BSEP INHIBITION			
All compounds (n=87)			
	$C_{\max,u}$ (μM)	MDD (mg/day)	Avg. dose (mg/day)
Non-cholestatic BSEP non-inhibitors (n=24)	0.30	310	85
Cholestatic BSEP non-inhibitors (n=26)	2.34	620	300
Non-cholestatic BSEP inhibitors (n=15)	0.08	312.5	217
Cholestatic BSEP inhibitors (n=22)	0.05	280	250
MRP4 INHIBITION			
All compounds (n= 87)			
	$C_{\max,u}$ (μM)	MDD (mg/day)	Avg. dose (mg/day)
Non-cholestatic MRP4 non-inhibitors (n=31)	0.25	240	81
Cholestatic MRP4 non-inhibitors (n=11)	13.2	1600	800
Non-cholestatic MRP4 inhibitors (n=8)	0.10	550	200
Cholestatic MRP4 inhibitors (n=37)	0.11	360	215
BSEP non-inhibitors (n=50)			
	$C_{\max,u}$ (μM)	MDD (mg/day)	Avg. dose (mg/day)
Non-cholestatic MRP4 non-inhibitors (n=20)	0.27	160	81
Cholestatic MRP4 non-inhibitors (n=10)	13.3	1600	580
Non-cholestatic MRP4 inhibitors (n=4)	0.27	950	143
Cholestatic MRP4 inhibitors (n=16)	0.28	400	280
BSEP inhibitors (n=37)			
	$C_{\max,u}$ (μM)	MDD (mg/day)	Avg. dose (mg/day)
Non-cholestatic MRP4 non-inhibitors (n=11)	0.05	200	288
Cholestatic MRP4 non-inhibitors (n=1)	0.01	2400	2100
Non-cholestatic MRP4 inhibitors (n=4)	0.16	257.5	123
Cholestatic MRP4 inhibitors (n=21)	0.06	200	125

If dosing was given as mg/kg or mg/m², the maximum and average dose was calculated based on an adult with 70 kg and a body surface area of 1.7.