SUPPORTING INFORMATION

Novel In Vitro Method Reveals Drugs that Inhibit Organic Solute Transporter Alpha/Beta (OSTα/β)

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Supporting Table 1. Inhibition of organic solute transporter α/β (OSTα/β)-mediated dehydroepiandrosterone sulfate (DHEAS) transport by 77 test compounds/fixed dose combinations, registered drug-induced liver injury (DILI) cases, liver injury causality of the test compounds and reported transporter inhibition.

Compound	OSTα/β Inhibition (% of control)			Cholestatic DILI cases[a]	Hepatotoxicity Likelihood[b]	Verified DILI causality[c]	Label Section[c]	Reported Inhibition[d]
	Mean	SEM	p-value					
DILIN dataset								
Vancomycin	119	11	ns	1	—	2	—	—
Regorafenib	116	3	ns	1	—	2	Warnings and precautions	ABCC3
Azithromycin	116	8	ns	2	A	2	Adverse reactions	ABCB1
Nicotinic Acid	115	3	ns	1	—	1	Warnings and precautions	—
Duloxetine	115	8	ns	3	B	1	Warnings and precautions	—
Sulfamethoxazole + Trimethoprim	112	9	ns	10	—	2	—	—
Meropenem	110	14	ns	2	D	2	Warnings and precautions	—
Rosuvastatin	108	3	ns	1	B	2	Warnings and precautions	ABCC3, ABCC4, ABCG2
Verapamil	108	11	ns	1	B	2	—	ABCB1, ABCC1, SLC22A1, SLC22A2, SLC22A4, SLC47A1, SLC47A2, SLCO1A2
Meloxicam	105	8	ns	2	—	2	Warnings and precautions	ABCB1
Sulindac	105	5	ns	1	—	1	Warnings and precautions	ABCC4, SLC22A6, SLC22A7, SLC22A8, SLC22A11
Methotrexate	104	7	ns	2	—	1	Box warning	ABCC5
Terbinafine	104	6	ns	2	B	1	Warnings and precautions	—
Metoclopramide	104	3	ns	1	—	2	Adverse reactions	—
Temozolomide	103	5	ns	3	—	2	Adverse reactions	—
Mercaptopurine	103	6	ns	3	A	1	Warnings and precautions	—
Trimethoprim	101	6	ns	*	—	2		SLC22A2
Penicillamine	101	8	ns	1	—	2	Warnings and precautions	—

Moxifloxacin	100	5	ns	2	—	2	Adverse reactions	—
Simvastatin	100	5	ns	1	A	2	Warnings and precautions	ABCB11, ABCG2, SLC22A1, SLC22A6, SLC22A8, SLCO2B1
Azathioprine	99	5	ns	1	A	1	Warnings and precautions	—
Minocycline	99	4	ns	2	A	1	Warnings and precautions	—
Ezetimibe	99	8	ns	*	C	2	Warnings and precautions	—
Nitrofurantoin	98	7	ns	5	—	1	Warnings and precautions	ABCC4
Methimazole	97	4	ns	1	—	1	—	SLC22A4
Phenytoin	97	5	ns	1	—	1	Warnings and precautions	—
Cefaclor	96	6	ns	1	—	2	Adverse reactions	SLC15A2
Cefotaxime	96	6	ns	1	—	2	Adverse reactions	ABCC4, SLC22A6, SLC22A7, SLC22A8, SLC22A11
Acyclovir	95	16	ns	1	D	2	Adverse reactions	—
Rifampicin	95	4	ns	1	—	1	Warnings and precautions	ABCB11, ABCC2, ABCC3, ABCC4, SLC22A6, SLCO1B1, SLCO1B3
Enalapril	94	8	ns	1	B	2	Adverse reactions	—
Cefazolin	94	9	ns	10	—	2	Adverse reactions	SLC22A6, SLC22A8, SLC22A11
Ceftriaxone	93	9	ns	1	—	2	—	ABCC4, SLC22A6, SLC22A8, SLC22A11
Ciprofloxacin	91	7	ns	4	B	1	—	—
Alfuzosin	90	8	ns	1	C	2	Adverse reactions	—
Allopurinol	89	11	ns	2	A	1	Warnings and precautions	—
Artemisinin	89	4	ns	1	D	—	—	—
Amoxicillin + Clavulanic Acid	89	9	ns	42	A	—	—	—
Carbamazepine	89	9	ns	1	A	1	Warnings and precautions	—
Ezetimibe + Simvastatin	88	7	ns	2	—	2	Warnings and precautions	—

Metaxalone	87	8	ns	1	E	2	Adverse reactions	—
Hydralazine	86	13	ns	2	—	2	Adverse reactions	—
Levofloxacin	86	4	ns	4	—	1	Warnings and precautions	—
Benazepril + Hydrochlorothiazide	86	7	ns	2	—	—	—	—
Sulfamethoxazole	86	15	ns	*	—	—	—	—
Amoxicillin	84	10	ns	3	B	2	Adverse reactions	SLC15A2
Clavulanic Acid	84	4	ns	*	—	—	—	—
Hydrochlorothiazide	83	7	ns	*	D	2	Adverse reactions	SLC22A6, SLC22A7, SLC22A8
Benazepril	83	11	ns	*	D	2	Adverse reactions	—
Doxycycline	81	3	ns	1	C	2	Adverse reactions	—
Estradiol	81	3	ns	1	—	2	Adverse reactions	—
Fenofibrate	81	4	ns	2	B	2	Warnings and precautions	ABCB11, SLCO1B1
Creatine	81	3	ns	1	—	—	—	SLC22A2**
Cefuroxime	80	5	ns	1	—	2	—	ABCC4
Oxaliplatin	79	12	ns	3	—	1	Warnings and precautions	—
Amiodarone	79	8	ns	1	A	1	Box warning	ABCB1
Lovastatin	79	4	ns	2	B	2	Warnings and precautions	ABCB11, ABCC3
Atorvastatin	65	5	<0.0005	4	A	1	Warnings and precautions	ABCB11, ABCC3, ABCC4, ABCG2, SLCO2B1
Norgestimate	59	5	<0.0001	*	—	2	Adverse reactions	—
Ethinylestradiol	36	5	<0.0001	*	—	3	Adverse reactions	ABCB11, ABCC3***
Ethinylestradiol + Norgestimate	32	3	<0.0001	1	—	2	Adverse reactions	—
Reported OSTα/β substrates or inhibitors								
Digoxin	95	6	ns	—	E	4	—	ABCB1, ABCB4, SLC51A/SLC51B, SLCO1B3

Estrone sulfate	88	6	ns	—	—	3	Adverse reactions	ABCC1, SLC51A/SLC51B, SLCO1B1, SLCO1B3
Sulfobromophthalein	86	6	ns	—	—	—	—	SLC10A1, SLC51A/SLC51B, SLCO1A2, SLCO1B1, SLCO1B3, SLCO2B1
Probenecid	80	3	ns	—	—	3	Adverse reactions	ABCC4, ABCC5, ABCC6, SLC22A6, SLC22A7, SLC22A8, SLC22A11, SLC22A12, SLCO1B3
Indomethacin	79	5	ns	—	—	1	Warnings and precautions	ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC6, SLC22A6, SLC22A7, SLC22A8, SLC22A11, SLC51A/SLC51B, SLCO1B1
Spironolactone	77	6	<0.05	—	D	2	Adverse reactions	SLC51A/SLC51B, SLCO1B1
Fidaxomicin	37	3	<0.0001	—	E	—	—	ABCC3, SLC51A/SLC51B
Bile acids								
Taurocholate	94	7	ns	—	—	—	—	ABCC3, SLC51A/SLC51B, SLCO1B1
Taurolithocholic acid sulfate	88	7	ns	—	—	—	—	SLC51A/SLC51B
Chenodeoxycholate	82	3	ns	—	—	—	—	SLC10A2, SLCO1B1
Glycochenodeoxycholate	73	7	<0.01	—	—	—	—	ABCB11, SLC10A2, SLC51A/SLC51B
DILI compounds								
Paroxetin	120	5	ns	—	—	2	Adverse reactions	—
Valproate	99	4	ns	—	—	1	Box warning	—
Bosentan	83	4	ns	—	C	1	Box warning	ABCB11, ABCC3, ABCC4, SLC10A1
Troglitazone	73	7	<0.01	—	—	1	Withdrawn	ABCB11, ABCC3, ABCC4, SLCO1B1, SLCO1B3
Troglitazone sulfate	64	4	<0.0001	—	—	—	—	ABCB1

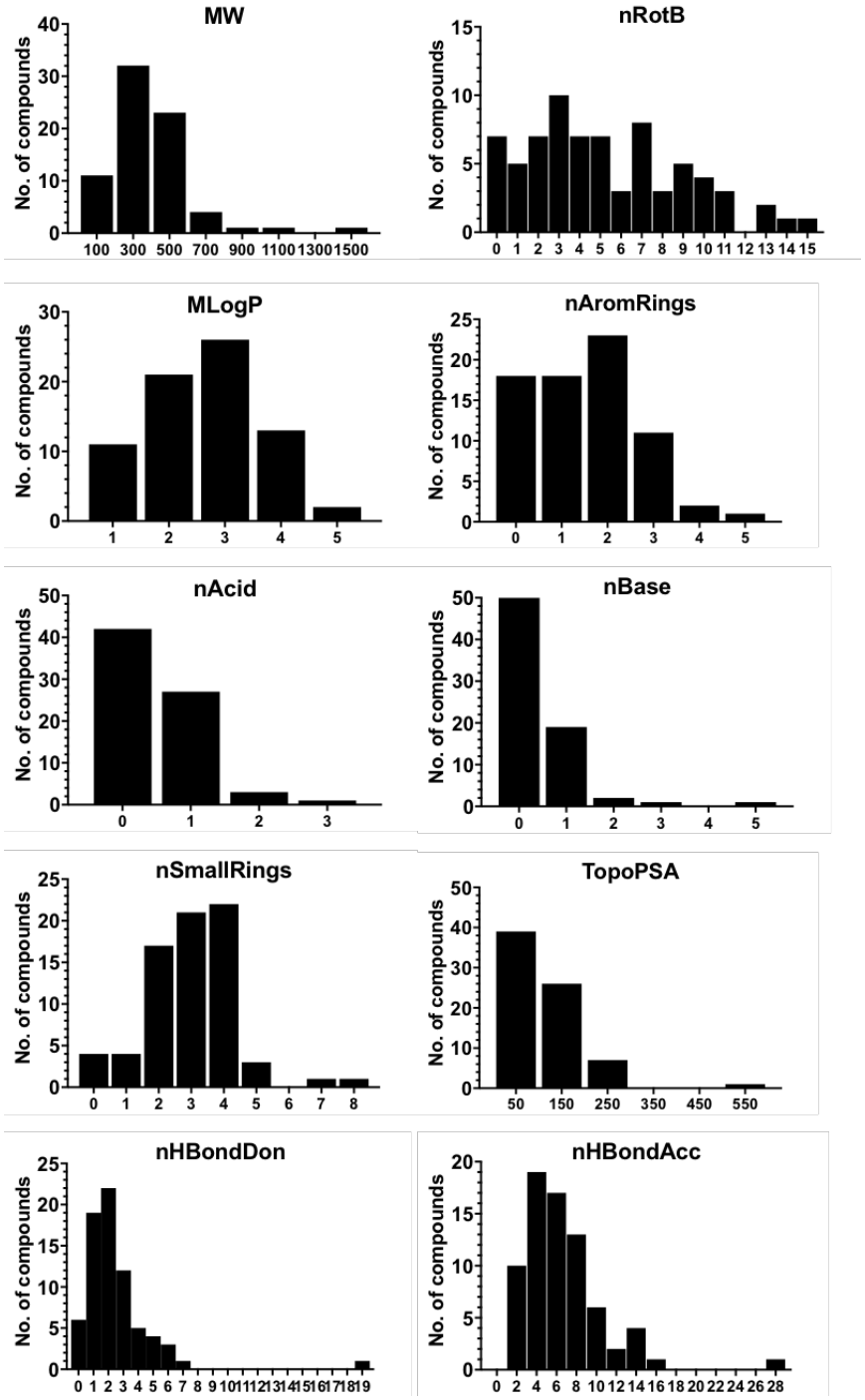
[a] causality and occurrence of compounds in the Drug-Induced Liver Injury Network (DILIN) dataset of 190 cholestatic patients (02.17.2017).

[b] hepatotoxicity likelihood categories defined in LiverTox. **A**, well known to cause liver injury, >50 cases reported; **B**, known to cause liver injury, 12-50 cases reported; **C**, probably causes liver injury, <12 cases reported; **D**, possible cause of liver injury; **E**, not believed to cause liver injury.[1]
[c] DILI causality and labeling of compounds: 1=most, 2=less, 3=ambiguous, 4=no.[2]
[d] transporter inhibition by test compounds.[3-7]
*, reported only as fixed dose combination; **, creatinine; *** ethinylestradiol glucuronide;
— not reported; ns, not significant; ABC, ATP-binding cassette; SLC, solute carrier transporter; SLCO, solute carrier organic anion transporter.

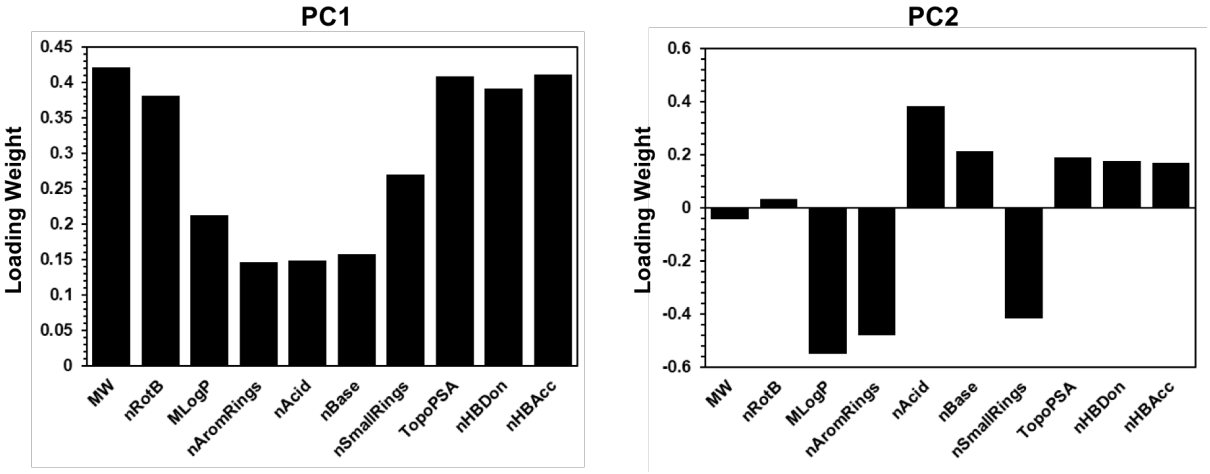
Supporting Table 2. Molecular characteristics of OSTα/β inhibitors identified in this study compared to the properties of compounds that did not inhibit OSTα/β. The molecular descriptors were calculated based on compound SMILES using the rcdk package in R (see details in Materials and Methods section).

	MW	nRotB	MLogP	nAromRings	nAcid	nBase	nSmallRings	TopoPSA	nHBDon	nHBAcc
Noninhibitors										
Mean	381	5	2.5	2	1	0	3	114	2	6
Median	361	4	2.6	2	0	0	3	90	2	5
Min	114	0	1.1	0	0	0	0	3	0	1
Max	1447	14	4.9	5	3	5	8	530	19	27
Identified OSTα/β inhibitors										
Atorvastatin	557	13	4.21	4	1	0	4	115	3	6
Ethinyl estradiol	296	0	3.44	1	0	0	4	40	2	1
Fidaxomicin	1058	15	4.98	1	0	0	3	267	7	16
Glycochenodeoxycholate	449	7	3.66	0	1	0	4	107	4	6
Norgestimate	369	3	3.55	0	0	0	4	59	1	2
Troglitazone	441	5	3.33	2	0	0	4	110	2	3
Troglitazone sulfate	543	7	2.78	2	1	1	4	165	1	6
Mean	531	7	3.7	1	0	0	4	123	3	6
Median	449	7	3.6	1	0	0	4	110	2	6
Min	296	0	2.8	0	0	0	3	40	1	1
Max	1058	15	5.0	4	1	1	4	267	7	16

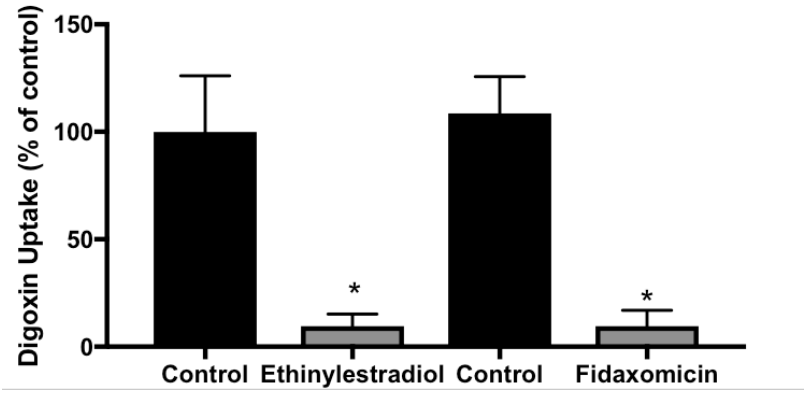
MW, molecular weight; nRotB, number of rotating bonds; MLogP, logP determined by Moriguchi's method; nAromRings, number of aromatic rings; nAcid, acidic group count; nBase, basic group count; nSmallRings, number of small rings; TopoPSA, topological polar surface area; nHBDon, number of hydrogen bond donors; nHBAcc, number of hydrogen bond acceptors.



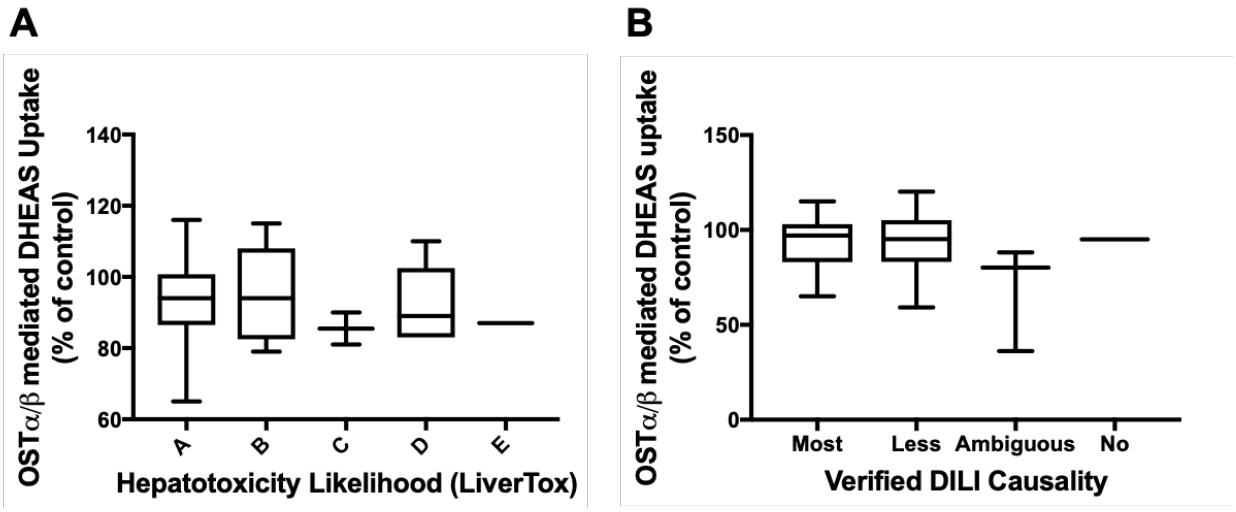
Supporting Figure 1. The frequency distribution of the molecular descriptors of the test compound dataset investigated for OSTα/β inhibition. The descriptors were calculated based on compound SMILES using the rcdk package in R (see details in Materials and Methods section). MW, molecular weight; nRotB, number of rotating bonds; MLogP, logP determined by Moriguchi's method; nAromRings, number of aromatic rings; nAcid, acidic group count; nBase, basic group count; nSmallRings, number of small rings; TopoPSA, topological polar surface area; nHBDon, number of hydrogen bond donors; nHBAcc, number of hydrogen bond acceptors.



Supporting Figure 2. The influence of descriptors, shown as loading weights, on the first two principal components (PC1 panel, PC2 panel) of the principal component analysis (PCA). The PCA was performed on the library of the tested compounds and FDA approved drugs included in the IDA2PM library (*n* = 1583). MW, molecular weight; nRotB, number of rotating bonds; MLogP, logP determined by Moriguchi's method; nAromRings, number of aromatic rings; nAcid, acidic group count; nBase, basic group count; nSmallRings, number of small rings; TopoPSA, topological polar surface area; nHBDon, number of hydrogen bond donors; nHBAcc, number of hydrogen bond acceptors.



Supporting Figure 3. The inhibitory effect of ethinylestradiol and fidaxomicin on OSTα/β-mediated digoxin uptake. OSTab and Mock cells were treated with a test compound either prior to (ethinylestradiol, 100 μM) or with (fidaxomicin, 100 μM) the probe substrate, [^{3}H]-digoxin (300 nCi/ml; 1 μM) in modified extracellular fluid (pH 7.4) at 37°C. Background levels derived from Mock cells were subtracted, and uptake measurements were normalized to total cell protein. Data are expressed as percentage of vehicle control (mean ± range) from one experiment. *, $p<0.05$.



Supporting Figure 4. OSTα/β-mediated uptake of DHEAS in the presence of the tested compounds versus their A) hepatotoxicity likelihood and B) verified DILI causality. Figure is based on the data from Supporting Table 1.

SUPPORTING REFERENCES

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