

Identification and Quantitative Assessment of Uremic Solutes as Inhibitors of Renal Organic Anion Transporters, OAT1 and OAT3

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Table S1 Summary of inhibitor screening

Uremic solute	Category	Tested Concentration (μ M)	Inhibition (%)	
			OAT1	OAT3
2-Heptenal	W	50	19 $\pm$ 3	24 $\pm$ 3
2-Hexenal	W	60	0 $\pm$ 3	-1 $\pm$ 5
2-Nonenal	W	72	72 $\pm$ 1	51 $\pm$ 2
2-Octenal	W	26	42 $\pm$ 5	36 $\pm$ 2
4-Decenal	W	74	75 $\pm$ 1	60 $\pm$ 4
8-OHdG	W	0.3	-4 $\pm$ 4	0 $\pm$ 6
Acrolein	P	150	-3 $\pm$ 3	5 $\pm$ 12
ADMA	W	420	8 $\pm$ 6	4 $\pm$ 6
Alpha-N-acetylarginine	W	2100	-4 $\pm$ 8	-22 $\pm$ 5
Anthranilic acid	W	12	3 $\pm$ 2	8 $\pm$ 9
Aribitol	W	22000	12 $\pm$ 2	2 $\pm$ 7
Carboxymethyllysine	P	2500	-5 $\pm$ 4	6 $\pm$ 6
Cholecystokinin	M	0.03	-7 $\pm$ 3	15 $\pm$ 13
CMPF	P	300	64 $\pm$ 2	94 $\pm$ 0
Creatine	W	50000	33 $\pm$ 4	22 $\pm$ 6
Creatinine	W	220000	89 $\pm$ 0	70 $\pm$ 3
Cysteine	W	56	-2 $\pm$ 3	9 $\pm$ 6
Decanal	W	64	17 $\pm$ 3	26 $\pm$ 4
Dihydroxyphenylalanine	P	1500	-19 $\pm$ 3	-62 $\pm$ 7
Dimethylamine	W	2500	-24 $\pm$ 3	-1 $\pm$ 5
Erythritol	W	7	-1 $\pm$ 3	11 $\pm$ 4
Ethylamine	W	150	16 $\pm$ 2	33 $\pm$ 2
Fibroblast growth factor-23	M	0.0005	-2 $\pm$ 13	7 $\pm$ 4
Gamma-guanidinobutyric acid	W	1100	5 $\pm$ 3	-1 $\pm$ 9
Glutathion, oxidized	M	18000	-1 $\pm$ 6	16 $\pm$ 4
Guanidine	W	150	-3 $\pm$ 3	10 $\pm$ 5
Guanidinoacetic acid	W	170	-4 $\pm$ 1	5 $\pm$ 6
Heptanal	W	54	27 $\pm$ 4	20 $\pm$ 6
Hexanal	W	52	3 $\pm$ 5	1 $\pm$ 4
Hippuric acid	P	15000	94 $\pm$ 1	83 $\pm$ 2
Homocysteine	P	3500	-1 $\pm$ 3	15 $\pm$ 5
Hypoxanthine	W	20	-2 $\pm$ 2	5 $\pm$ 3
IL-6	M	0.00004	0 $\pm$ 16	4 $\pm$ 3
IL-8	M	0.0005	1 $\pm$ 3	6 $\pm$ 3
IL10	M	0.06	-2 $\pm$ 6	-6 $\pm$ 4
Indole acetate	P	250	74 $\pm$ 2	38 $\pm$ 6

Indoxyl sulfate	P	2000	86 ± 2	91 ± 1
Indoxyl-beta-d-glucuronide	P	950	32 ± 2	59 ± 5
Kynurenic acid	P	80	70 ± 2	91 ± 0
Leptin	M	0.4	-13 ± 3	4 ± 4
Malondialdehyde	W	1000	8 ± 1	8 ± 4
Manitol	W	42	-14 ± 4	7 ± 10
Melatonin	P	0.35	-7 ± 1	5 ± 2
Met-Enkephalin	M	0.003	8 ± 5	7 ± 3
Methylamine	W	1900	8 ± 3	21 ± 3
Methylguanidine	W	200	-17 ± 5	1 ± 3
N2,N2-Dimethylguanosine	W	140	0 ± 2	52 ± 2
t6-ADO	W	180	-3 ± 3	20 ± 3
Neopterin	W	8	-5 ± 1	-5 ± 6
Nicotinamide	W	30	-3 ± 1	-4 ± 4
Nonanal	W	50	72 ± 1	42 ± 1
Noradrenalin	W	1.2	-13 ± 4	4 ± 6
Orotic acid	W	900	20 ± 1	8 ± 6
Oxalate	W	4200	-4 ± 1	4 ± 7
p-Cresyl sulfate	P	1400	77 ± 1	84 ± 3
Pentosidine	P	30	-5 ± 1	-3 ± 3
Phenol	P	6400	31 ± 1	57 ± 0
Phenylacetic acid	W	175000	99 ± 0	93 ± 2
PTH	M	0.02	4 ± 1	5 ± 3
Putrescine	P	10	26 ± 4	41 ± 3
Quinolinic acid	P	4	8 ± 5	11 ± 9
Resistin	M	0.4	5 ± 7	-13 ± 5
S-Adenosylhomocysteine	W	80	-12 ± 8	-12 ± 8
Sorbitol	W	4000	-1 ± 2	12 ± 6
Spermidine	P	70	-12 ± 6	4 ± 5
Spermine	P	0.9	2 ± 2	2 ± 7
Thiocyanate	P	32000	-8 ± 2	12 ± 3
TNF-a	M	0.0002	0 ± 8	6 ± 7
Trimethylamine	W	140	-5 ± 4	-2 ± 3
Trimethylamine-N-oxide	W	100	-11 ± 4	-3 ± 8
Uric acid	W	2000	78 ± 1	91 ± 1
VEGF	M	0.001	3 ± 10	7 ± 6

M, middle molecule; P, protein bound solute; W, free water soluble solute; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CMPF, 3-Carboxy-4-methyl-5-propyl-2-furan-propanoic acid; IL-6, interleukin 6; IL-8, Interleukin 8; IL-10, Interleukin 10; PTH, parathyroid hormone; t6-Ado, N6-Carbamoylthreonyladenosine; TNFa, tumor necrosis factor alpha

Table S2 Summary of CL_{sec}/GFR in normal and different stages of CKD

	CL_{sec}/GFR				
	Normal	CKD			
		Stage 2	Stage 3	Stage 4	Stage 5
Adefovir ¹	0.98 (7)	1.30 (8)	1.20 (7)	0.98 (10)	
Amoxicillin ²	0.54 (6)	N/A	0.62 (6)	0.33 (6)	0.36 (5)
Avibactam ³	0.80 (6)	0.25 (6)	0.32 (6)	0.02 (6)	
Cefotaxime ⁴	0.62 (7)	1.53 (7)	0.42 (7)	0.28 (4)	0.98 (5) ^b
Cefazolin ⁵	0.28 (7)	0.55 (1)	0.38 (2)	0.19 (3)	0.26 (3)
Ceftibuten ⁶	0.15 (6)	0.09 (6)	0.05 (6)	0.06 (6)	0.43 (6) ^b
Ceftizoxime ⁷	0.43 (11)	0.44 (10)	0.44 (5)	N/A ^a	0.79 (3) ^b
Ciprofloxacin ⁸	2.56 (6)	1.35 (1)	2.83 (5)	1.20 (3)	1.32 (6)
Entecavir ⁹	2.53 (6)	2.31 (6)	2.62 (6)	0.86 (6)	
Famotidine ¹⁰	2.26 (7)	2.77 (9)	2.38 (5)	1.23 (10)	
Ganciclovir ¹¹	1.61 (8)	1.39 (6)	0.72 (6)	0.70 (6)	
Hydrochlorothiazide ¹²	2.07 (6)	0.73 (6)	0.71 (7)	0.34 (1)	0.54 (1)
Pemetrexed ¹³	0.38 (18)	0.22 (13)	0.17 (15)	0.03 (1)	
Quinaprilat ¹⁴	0.39 (5)	0.36 (5)	0.23 (6)	0.14 (2)	0.14 (2)
Ro 64-0802 ¹⁵	1.46 (5)	0.63 (5)	0.60 (5)	0.20 (5)	
Sitagliptin ¹⁶	2.31 (82)	3.08 (6)	2.51 (6)	2.10 (6)	
Tazobactam ¹⁷	0.99 (11)	1.71 (6)	1.33 (7)	0.54 (6)	
Tenofovir ¹⁸	1.91 (3)	1.67 (10)	1.81 (8)	0.79 (11)	

Numbers in parentheses represent number of patients enrolled. CL_{sec} is secretory clearance. It was acquired by the equation: $CL_{sec} = CL_r - fu * GFR$ where CL_r is renal clearance, fu is unbound fraction and GFR is glomerular filtration rate. N/A, not available; ^aThe calculated value was negative and was not shown; ^bPatients were on dialysis.

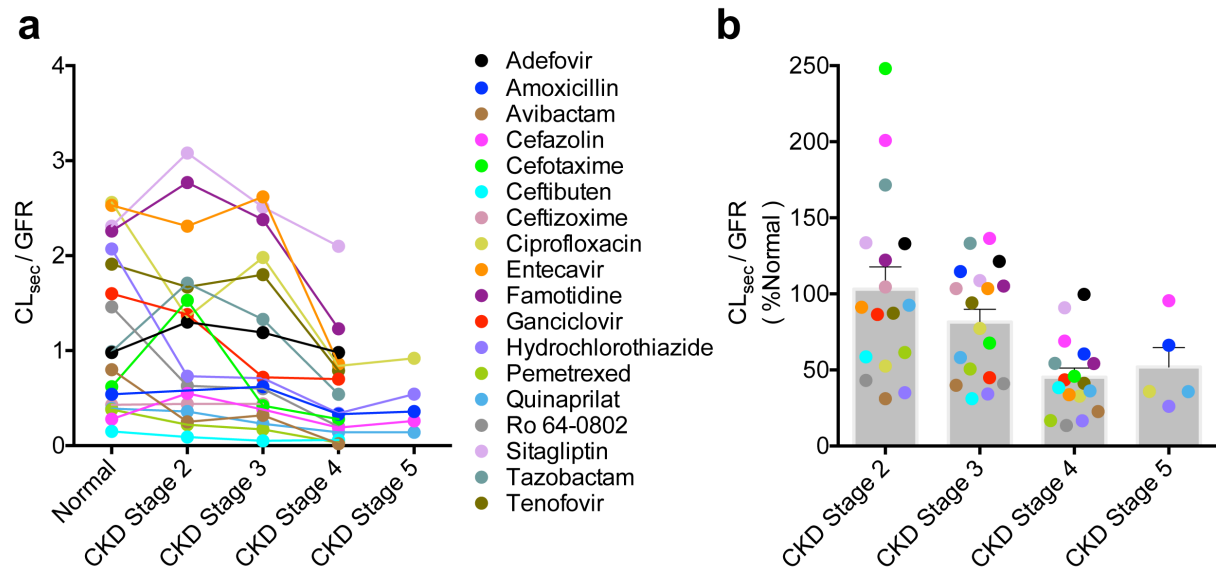


Figure S3 Graphic view of secretory clearance (CL_{sec}) over GFR in normal and different stages of CKD. Raw data (a) and normalized data (b) are shown. CKD stage 5d is not included here.

Table S4 Uremic solutes as potential in vivo substrates of OAT1 and OAT3

	Category	[I] (μM) ^a	OAT1			OAT3		
			[I]/IC ₅₀ ^b	KO / WT ^c	Km (μM) ^d	[I]/IC ₅₀ ^b	KO / WT ^c	Km (μM) ^d
CMPF	P	37	0.5		140 ¹⁹	1.3		27 ¹⁹
Creatinine	F	1,200	< 0.1	1.2 ^{f, 20}		< 0.1	2.0 ^{f, 21}	
Hippuric Acid	P	290	9.4	N/A ^{f, 22}	24 ¹⁹	7.1		
Indole Acetate	P	2.5	< 0.1		14 ¹⁹	N/A ^e		
Indoxyl Sulfate	P	21	0.2	9.4 ^{f, 22}	21 ¹⁹	< 0.1		260 ¹⁹
Kynurenic Acid	P	0.8	< 0.1		5 ²³	< 0.1		5 ²³
N2,N2-Dimethylguanosine	F	1.3	N/A ^e	0.5 ^{g, 22}		< 0.1		
Orotic Acid	F	220	N/A ^e	0.6 ^{g, 22} ; 0.5 ^{g, 20}		N/A ^e		
p-Cresyl Sulfate	P	21	0.1		130 ²⁴	0.1		N/A ²⁴
Uric Acid	F	500	0.2	0.7 ^{g, 20}	940 ²⁵	0.8	0.7 ^{g, 26}	380 ²⁷

CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; W, free water soluble solutes; P, protein bound solutes; IC₅₀, half maximal inhibitory concentration; [I]/ IC₅₀, ratio of highest concentration in CKD over IC₅₀; N/A, not available; ^aHighest unbound concentrations in CKD; total concentrations are shown for CMPF and kynurenic acid as unbound concentrations are not available; ^bData from this study; ^cMetabolomics studies in mice. KO, transporter knock-out mice; WT wild-type mice; ^dMichaelis-Menten constant acquired from human transporter overexpressing cells; ^eNot a designated inhibitor in this study; ^fRatio of plasma concentrations; ^gRatio of urine concentrations

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