Supporting Information

Structural Requirements of the ASBT by 3D-QSAR Analysis Using Aminopyridine Conjugates of Chenodeoxycholic Acid

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Running Title: conjugate QSAR for ASBT transporter

Table S1. Physicochemical characteristics of conjugates.

Comp.	LC-MS/MS					^{1}H-NMR (DMSO-*d*6)[d]
	Exact Mass [a]	Q1 Mass (m/z) [b]	Q3 Mass (m/z) [b]	Retention Time (min)	X-Initial B% in gradient[c]	
1	597.38	598.30	94.90	1.04	35%	12.52 (1H, s, β), 10.44 (1H, s, γ), 8.29 (1H, d, α), 8.06 (1H, d, α), 8.00 (1H, d, γ), 7.75 (1H, t, α), 7.07 (1H, t, α), 4.17 (1H, m, θ)
2	597.38	598.41	221.00	1.24	40%	12.34 (1H, s, β), 10.21 (1H, s, γ) 8.74 (1H, s, α) 8.26 (1H, d, α), 8.05 (1H, d, α), 8.00 (1H, d, γ) 7.35 (1H, t, α), 4.20 (1H, m, θ)
3	597.38	598.41	221.00	2.24	30%	12.63 (1H, s, β), 10.55 (1H, s, γ), 8.45 (2H, d, α), 8.09 (1H, d, γ), 7.66 (2H, d, α), 4.23 (1H, m, θ)
4	625.41	626.31	189.01	1.23	33%	12.50 (1H, s, β), 8.74 (1H, d, 7), 8.22 (1H, t, α), 8.09 (1H, d, γ), 8.05 (1H, t, γ), 7.72 (1H, t, α) 7.25 (1H, d, α), 4.12 (1H, m, θ) 3.52 (1H, m, α) 3.09 (1H, t, α)
5	611.39	612.30	238.03	1.55	35%	12.41 (1H, s, β), 9.45 (1H, s, γ), 8.23 (1H, d, α), 8.08 (1H, d, γ), 7.78 (1H, d, α), 7.20 (1H, t, α), 4.22 (1H, m, θ), 2.39 (3H, s, α)
6	611.39	612.25	238.03	1.00	40%	12.43 (1H, s, β), 9.51 (1H, s, γ), 8.49 (1H, s, α), 8.23 (1H, d, α) 8.09 (1H, d, γ) 7.24 (1H, d, α), 4.25 (1H, m, θ), 2.19 (3H, s, α)
7	611.39	612.45	108.91	1.43	40%	12.21 (1H, s, β), 10.08(1H, s, γ), 8.50 (1H, s, α), 8.03 (1H, d, γ), 8.07 (1H, s, α), 7.87 (1H, s, α), 4.31 (1H, m, θ), 2.40 (3H, s, α)
8	611.39	612.25	237.99	1.38	35%	12.01 (1H, s, β), 10.03 (1H, s, γ), 8.57 (1H, s, α), 8.02 (1H, d, γ), 7.88 (1H, d, α), 7.16 (1H, d, α) 4.21 (1H, m, θ), 2.38 (3H, s, α)
9	611.39	612.30	108.97	1.98	32%	12.03 (1H, s, β), 10.24 (1H, s, γ), 8.26 (1H, d, α), 8.03 (1H, d, γ), 7.43 (1H, s, α), 7.34 (1H, d, α) 4.31

						(1H, m, θ) 2.39(3H, s, α)
10	625.41	626.81	234.04	1.46	35%	11.57 (1H, s, β) 10.31 (1H, s, γ), 7.89 (1H, d, γ), 7.75 (1H, s, α), 6.78 (1H, s, α), 4.27 (1H, m, θ) 2.32 (3H, s, α), 2.24 (3H, s, α)
11	715.46	716.54	NA	NA	NA	10.30 (1H, s, γ), 8.20 (1H, d, γ), 7.74 (1H, s, α), 7.35 (5H, s, β), 6.78 (1H, s, α), 5.09 (2H, s, β), 4.29 (1H, m, θ), 2.33 (3H, s, α), 2.24 (3H, s, α)
12	625.41	626.41	123.00	1.10	32%	11.48 (1H, s, β), 10.01 (1H, s, γ), 7.71 (1H, d, α), 7.43 (1H, d, γ), 7.01 (1H, d, α), 4.11 (H, m, θ), 2.35 (3H, s, α), 2.37 (3H, s, α)
13	715.46	716.57	NA	NA	NA	9.38 (1H, s, γ), 8.25 (1H, d, γ), 4.31 (1H, m, θ), 7.60 (1H, d, α), 7.35 (5H, s, β), 7.03 (1H, d, α), 5.12 (2H, s, β), 2.38 (3H, s, α), 2.31 (3H, s, α)
14	627.39	628.40	124.92	1.39	45%	12.40 (1H, s, β), 9.92 (1H, s, γ), 8.33 (1H, s, **7**), 8.04 (1H, d, γ), 7.87 (1H, d, α), 6.77 (1H, d, α), 4.19 (1H, m, θ), 3.80 (3H, s, α)
15	612.39	613.40	220.50	1.76	35%	12.56 (1H, s, β), 10.29 (1H, s, γ), 8.12 (1H, d, γ), 7.52 (1H, t, α), 7.31 (2H, s, α) 6.34 (1H, d, α), 5.93 (1H, d, α), 4.24 (1H, m, θ)
16	613.37	614.40	221.97	1.18	40%	12.59 (1H, s, β), 10.25 (1H, s, γ), 8.09 (1H, d, γ), 7.90 (1H, d, α), 7.30 (1H, d, α), 7.18 (1H, t, α) 5.47 (1H, s, α), 4.18 (1H, m, θ)
17	703.42	704.41	312.07	2.55	48%	10.20 (1H, s, γ), 8.25 (1H, d, γ), 7.88 (1H, d, α), 7.35 (5H, s, β), 7.27 (1H, d, α), 7.14 (1H, t, α), 5.11 (2H, s, β), 4.30 (1H, m, θ)
18	615.37	616.40	112.91	1.36	45%	12.50 (1H, s, β), 9.93 (1H, s, γ), 8.41 (1H, d, α), 8.06 (1H, d, γ), 7.92 (1H, d, α), 7.33 (1H, t, α), 4.19 (1H, m, θ)
19	615.37	616.41	112.98	1.52	40%	12.48 (1H, s, β), 10.70 (1H, s, γ), 8.58 (1H, s, α), 8.26 (1H, s, α), 8.13 (1H, d, γ), 8.08 (1H, s, α), 4.21 (1H, m, θ)

20	615.37	616.40	224.80	1.59	40%	12.57 (1H, s, β), 10.25 (1H, s, γ), 8.42 (1H, s, α), 8.17 (1H, d, α), 8.11 (1H, d, γ), 7.17 (1H, d, α), 4.34 (1H, m, θ)
21	675.29	676.3/678.30	599.40	4.73	45%	12.56 (1H, s, β), 10.35 (1H, s, γ), 9.35 (1H, s, α), 8.09 (1H, d, γ), 8.63 (1H, s, α), 8.36 (1H, s, α), 4.24 (1H, m, θ)
22	675.29	676.3/678.26	172.81	5.69	45%	12.54 (1H, s, β), 10.27 (1H, s, γ), 8.57 (1H, s, α), 8.09 (1H, d, γ), 7.96 (1H, d, α), 7.56 (1H, d, α), 4.23 (1H, m, θ)
23	631.34	632.30/634.30	540.16	4.87	40%	12.56 (1H, s, β), 10.29 (1H, s, γ), 8.58 (1H, s, α), 8.10 (1H, d, γ), 8.06 (1H, d, α), 7.45 (1H, d, α) 4.24 (1H, m, θ)
24	631.34	632.30/634.40	128.92	4.45	43%	12.51 (1H, s, β), 11.45 (1H, s, γ), 8.84 (1H, s, α), 8.68 (1H, d, α), 8.18 (1H, d, γ), 8.04 (1H, d, α), 4.22 (1H, m, θ)
25	612.38	613.40	221.12	2.72	35%	12.49 (1H, s, β), 9.76 (1H, s, γ), 8.06 (1H, s, γ), 7.66 (1H, d, α), 6.93 (1, t, α), 6.84 (1H, d, α), 6.75 (1H, t, α), 4.30 (1H, m, θ)
26	612.38	613.30	220.94	1.83	40%	12.58 (1H, s, β), 9.82 (1H, s, γ), 8.07 (1H, d, γ), 7.21 (1H, s, α), 7.08 (1H, t, α), 6.97 (1H, d, α), 6.46 (1H, d, α), 4.15 (1H, m, θ)
27	612.38	613.31	220.81	1.44	45%	12.53 (1H, s, β), 9.63 (1H, s, γ) 8.06 (1H, d, γ), 7.34 (2H, d, α), 6.68 (2H, d, α), 4.20 (1H, m, θ)

[a] Calculated monoisotopic mass.

[b] Q1 and Q3 mass are experimental mass $[M+H]^+$.

[c] The mobile phase was A, 0.1% formic acid in water; and B, 0.1% formic acid in acetonitrile. The gradient times were listed as percent organic phase, B%. 0–0.2 (X%), 0.2–3.0 (X–95%), 3.0–4.0 (95%), 4.0–4.2 (95-X%), and 4.2–7.0 min (X%)

[d] Only characteristic peaks are shown, CDCA region and methylene peaks are not shown; values are in ppm relative to TMS; β indicates assignment to proton peaks in R_1; α indicates assignment to protons in R_2; γ indicates assignment to protons in amide, θ indicates the proton in α carbon.

Table S2. 3D-QSAR models for inhibition and transport efficiency.

Comp.	Obs. pK_i[a]	Pre. pK_i		Obs. Log(J_{max}/K_t)[b]	Pre. Log(J_{max}/K_t)
		CoMFA-1	CoMSIA-3		CoMFA-2
1	5.28	5.26	5.29	0.790	0.794
2[c]	5.82	5.09	4.93	1.45	0.479
3	5.06	5.02	5.00	0.300	0.307
4	5.15	5.10	5.23	0.280	0.279
5	5.33	5.36	5.42	-0.150	-0.151
6	4.96	5.01	4.91	-0.410	-0.412
7	4.63	4.62	4.71	0.290	0.289
8	5.1	5.31	5.20	0.0800	0.0770
9	5.55	5.57	5.43	0.300	0.298
10	5.18	5.11	5.17	0.980	0.984
11	3.91	3.94	4.00	ND	ND
12	5.72	5.58	5.70	-0.260	-0.257
13	4.55	4.50	4.51	ND	ND
14	5.05	5.05	5.11	1.31	1.31
15	5.08	5.10	5.10	1.51	1.51
16	5.51	5.55	5.48	1.16	1.16
17	4.75	4.78	4.66	NM	NM
18[c]	5.24	5.09	5.11	3.43	0.783
19	5.16	5.09	5.00	0.920	0.925
20	5.24	5.09	5.12	0.420	0.418
21	5.3	5.09	5.36	0.310	0.308
22[c]	4.68	4.74	4.67	0.620	0.331
23	5.1	5.10	5.25	NM	NM
24	5.1	5.15	5.13	NM	NM
25[c]	5.45	5.27	5.47	1.42	0.649
26	5.61	5.65	5.66	1.15	1.15
27	5.14	5.20	5.12	0.460	0.457

[a] –log(K_i) calculated from observed K_i.

[b] Calculated from log($normJ_{max}/K_t \times 100$)

[c] **2**, **18**, **22**, and **25** were used as test set.

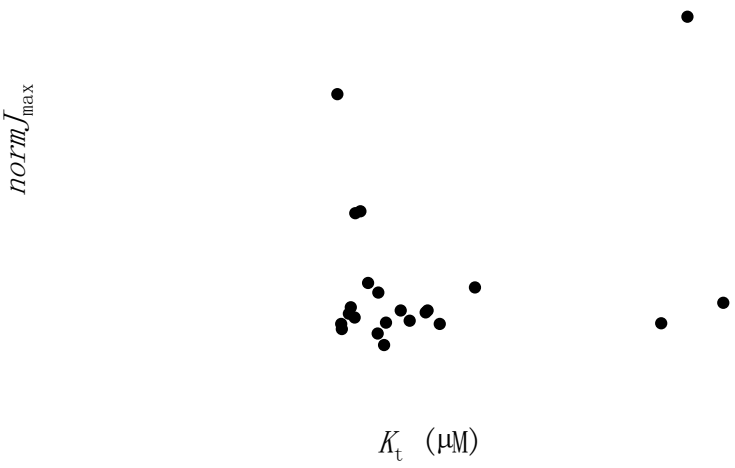

Figure S1. Relationship between $normJ_{max}$ and K_t. Transporting capacity was not associated with transporter affinity. Linear regression showed a slope of 0.008 μM^{-1} and r^2 of 0.064. **15**, **18**, **22**, and **25** yielded J_{max} higher than taurocholate. All other conjugates exhibited J_{max} less than taurocholate J_{max}, and ranged from 0.0791 to 0.716.

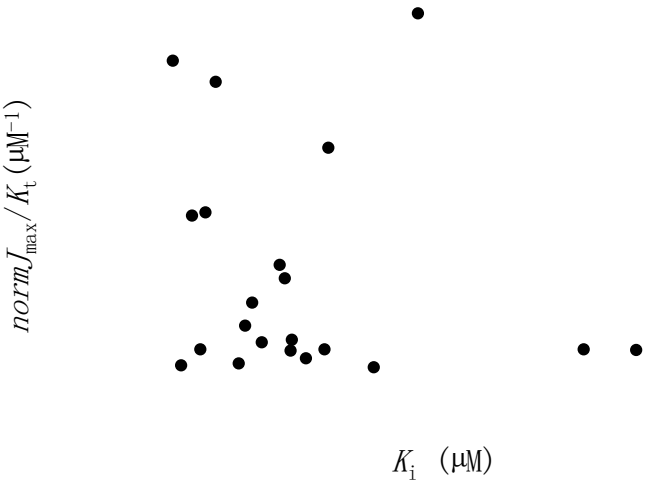

Figure S2. Relationship between $normJ_{max}/K_t$ and K_t. Linear regression showed a slope of -0.003 μM^{-2} and r^2 of 0.028. Transporting efficiency was not associated with inhibition potency. **18** was removed from the plot due to its high $normJ_{max}/K_t$ value of 26.7.