FOOD ADDITIVES AS INHIBITORS OF INTESTINAL DRUG TRANSPORTER OATP2B1

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SUPPORTING INFORMATION

Figure S1: Time-dependent uptake of DBF and estrone sulfate
Tables S1 and S2: Data used to analyze concentration-dependent uptake of DBF and estronesulfate
Figure S2: DBF fluorescence interference with food additives (50 µM)
Figure S3: Solubility of food additives (50 µM) in transport buffer
Figure S4: Structural formulas of the food additives that were identified as OATP2B1 inhibitors
Table S3: Molecular properties of the tested food additives
Table S4: Inhibition of OATP2B1-mediated uptake by food additives (50 µM) and comparison with results from study by Zou et al.[1]

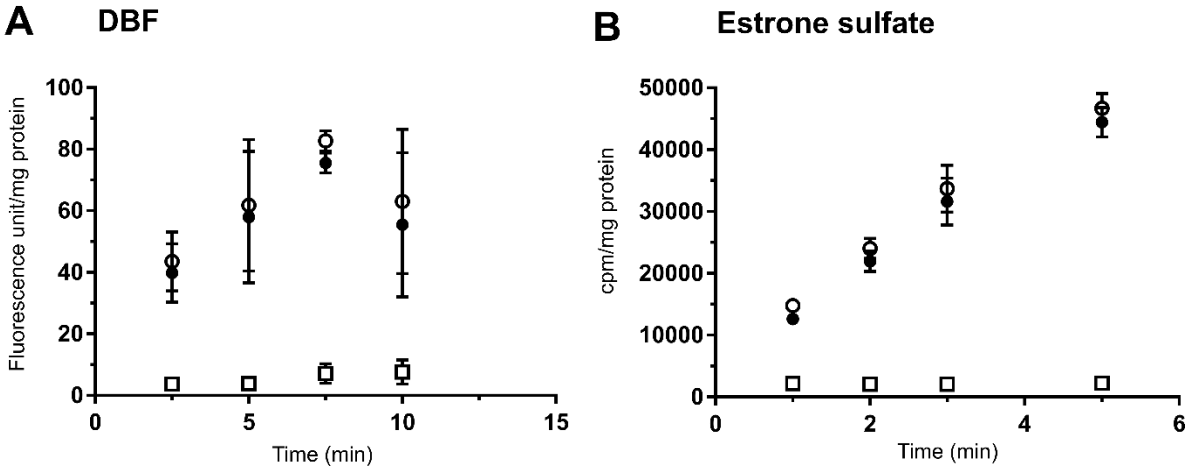


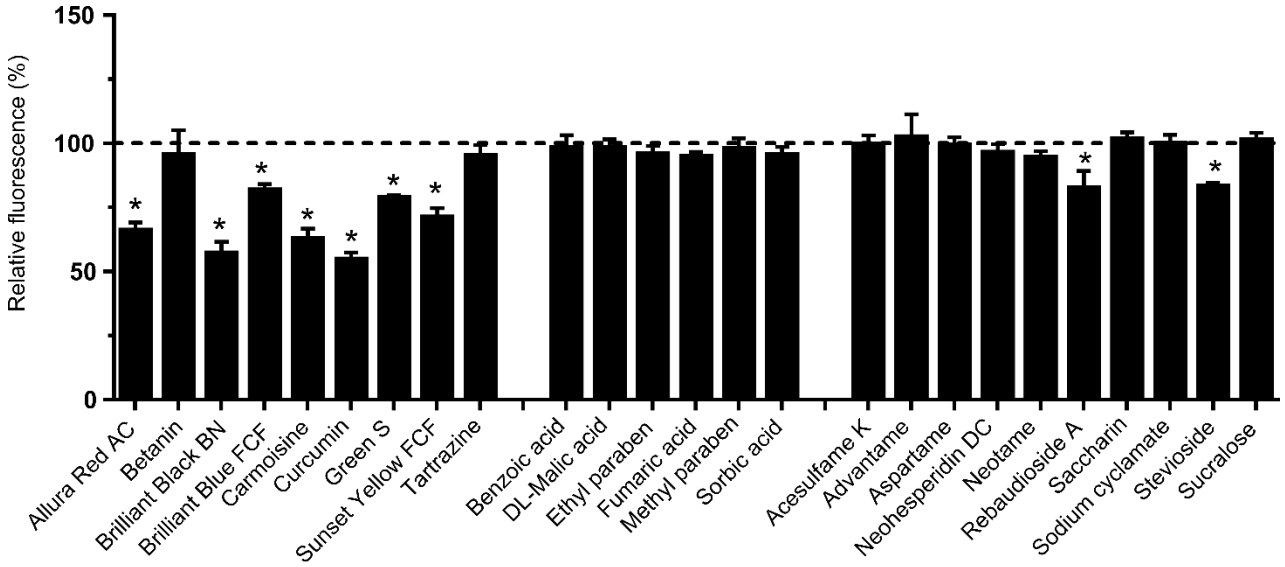
Figure S1. Time-dependent uptake of (A) 1 µM dibromofluorescein (DBF) and (B) 0.5 µM estrone sulfate into HEK293 cells expressing OATP2B1 (○) or control (□). OATP2B1-mediated transport (●) was obtained by subtracting control uptake from OATP2B1 uptake. Uptake of DBF and estrone sulfate were studied at pH 7.4 and pH 5.5, respectively. The data is presented as mean ± SD from one study with three replicate wells.

Table S1. Data used to analyze concentration-dependent uptake of DBF into OATP2B1 and control (eYFP) expressing HEK293 cells. OATP2B1-mediated uptake was obtained by subtracting control uptake from OATP2B1 uptake. The data is presented as mean ± SD from one study with three replicate wells.

DBF concentration (μM)	**OATP2B1 uptake** (fluorescence unit/ mg total protein/min)	**Control (eYFP) uptake** (fluorescence unit/ mg total protein/min)	**OATP2B1-mediated uptake** (fluorescence unit/ mg total protein/min)
0.01	1.72 ± 0.91	1.15 ± 0.68	0.56 ± 1.13
0.1	13.89 ± 0.31	1.60 ± 0.93	12.29 ± 0.98
0.5	54.13 ± 6.53	1.43 ± 0.24	52.70 ± 6.53
2.5	171.65 ± 23.15	5.30 ± 0.84	166.36 ± 23.17
5	202.29 ± 18.00	7.37 ± 0.75	194.92 ± 18.01
15	303.79 ± 16.36	25.17 ± 0.44	278.61 ± 16.37
30	356.22 ± 15.50	58.85 ± 9.98	297.37 ± 18.43

Table S2. Data used to analyze concentration-dependent uptake of estrone sulfate into OATP2B1 and control (eYFP) expressing HEK293 cells. OATP2B1-mediated uptake was obtained by subtracting control uptake from OATP2B1 uptake. The data is presented as mean ± SD from one study with three replicate wells.

Estrone sulfate concentration (μM)	**OATP2B1 uptake** (pmol/mg total protein/min)	**Control (eYFP) uptake** (pmol/mg total protein/min)	**OATP2B1-mediated uptake** (pmol/mg total protein/min)
0.1	0.63 ± 0.09	0.10 ± 0.02	0.53 ± 0.09
0.5	9.52 ± 0.18	0.65 ± 0.23	8.88 ± 0.18
1	23.66 ± 4.61	1.12 ± 0.06	22.54 ± 4.61
5	127.10 ± 18.58	4.77 ± 0.54	122.33 ± 18.58
15	310.56 ± 83.48	8.71 ± 1.53	301.85 ± 83.48
30	388.16 ± 54.89	17.37 ± 4.78	370.78 ± 54.89
50	486.40 ± 69.01	40.15 ± 18.11	446.24 ± 69.01
80	508.95 ± 80.73	84.26 ± 32.57	424.69 ± 80.73



* p<0.05, compared to control

Figure S2. Interference of food additives with dibromofluorescein (DBF) fluorescence assuming that food additives are completely retained in the final fluorescence sample. DBF (0.1 µM) fluorescence was measured with 50 µM food additive in 0.1 M NaOH. Results are expressed as mean ± SD of three replicate measurements normalized to control without food additive.

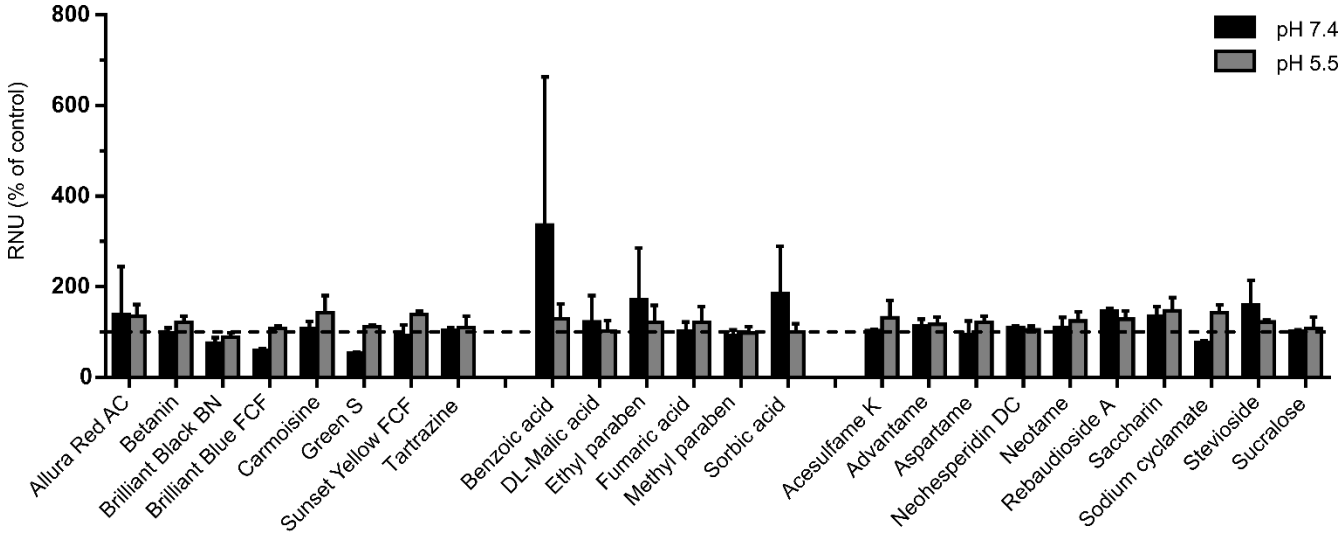
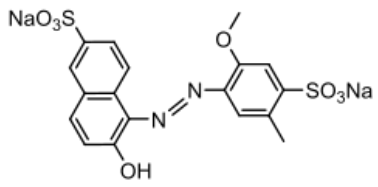
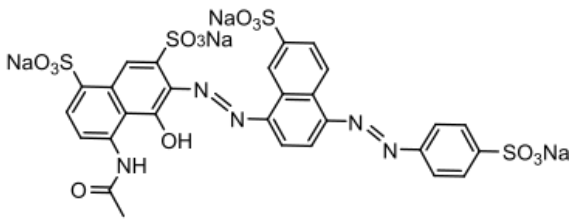


Figure S3. Solubility of food additives (50 µM) in transport buffer pH 7.4 or pH 5.5 measured with nephelometry. Results are expressed as mean ± SD (three replicates) relative nephelometric unit (RNU) of food additives in transport buffer normalized to RNU of transport buffer alone.

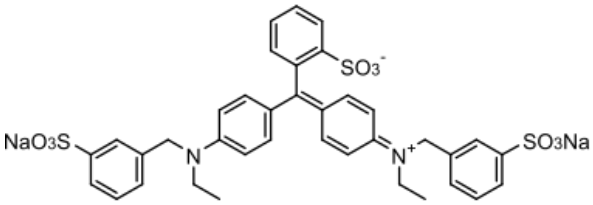
ALLURA RED AC



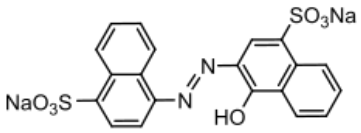
BRILLIANT BLACK BN



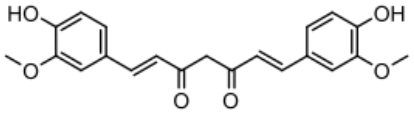
BRILLIANT BLUE FCF



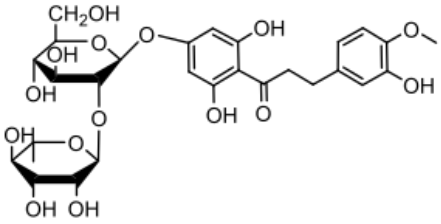
CARMOISINE



CURCUMIN



NEOHESPERIDIN DC



SUNSET YELLOW FCF

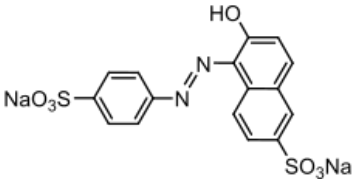


Figure S4. Structural formulas of the food additives that were identified as OATP2B1 inhibitors.

Table S3. Molecular properties of the tested food additives. The properties were calculated with ACD/Labs version 8.0 (Advanced Chemistry Development, Inc., Toronto, ON, Canada) for the parent compound.

Food additive	**Catalog number**	**E number**	**MW** (g/mol)	**PSA**	**$LogD_{7.4}$**	**$LogD_{5.5}$**
Acesulfame K (potassium salt)	47134	E 950	163	81	-2.32	-2.32
Advantame (monohydrate)	80054	E 969	459	134	-0.25	-0.03
Allura Red AC (sodium salt)	38213	E 129	452	180	-1.64	-1.64
Aspartame	47135	E 951	294	119	-1.49	-1.34
Benzoic acid	33047	E 210	122	37	-0.98	0.58
Betanin (red beet extract diluted with dextrin)	CDS000584	E 162	551	247	-6.59	-6.42
Brilliant Black BN (sodium salt)	11220	E 151	780	350	-7.64	-7.64
Brilliant Blue FCF (sodium salt)	80717	E 133	750	195	-0.18	-0.17
Carmoisine (sodium salt)	52245	E 122	458	170	-3.1	-3.07
Curcumin	C1386	E 100	368	93	2.84	2.92
DL-Malic acid	240176	E 296	134	95	-5.99	-3.9
Ethylparaben	111988	E 214	166	47	2.34	2.39
Fumaric acid	47910	E 297	116	75	-4.75	-3.09
Green S (sodium salt)	06737	E 142	556	152	0.98	1.25
Methylparaben	H5501	E 218	152	47	1.81	1.86
Neohesperidin dihydrochalcone	75041	E 959	613	245	2.59	3.08
Neotame	49777	E 961	378	105	1.07	1.17
Rebaudioside A	01432	E 960	967	374	-1.12	-1.12
Saccharin	109185	E 954	183	72	-1.09	-1.08
Sodium cyclamate (sodium salt)	47827	E 952	179	75	-2.52	-2.4
Sorbic acid	S1626	E 200	112	37	-1.41	0.39
Stevioside (hydrate)	S3572	E 960	805	295	1.19	1.19
Sucralose	PHR1342	E 955	398	129	0.68	0.68
Sunset Yellow FCF (sodium salt)	465224	E 110	408	170	-3.3	-3.3
Tartrazine (sodium salt)	03322	E 102	468	220	-6.63	-6.63

MW, molecular weight; PSA, polar surface area

Table S4. Inhibition of OATP2B1-mediated uptake of dibromofluorescein (DBF; 1 µM) at pH 7.4, and estrone sulfate (ES; 0.5 µM) at pH 5.5 by food additives at 50 µM concentration. Results are expressed as mean ± SD of OATP2B1-mediated uptake relative to control with DBF or estrone sulfate alone from one experiment with three replicates. For comparison, results from the study by Zou et al.[1] are presented.

Food additive	**DBF uptake (pH 7.4)** (% of control)	**ES uptake (pH 5.5)** (% of control)	**Zou et al.[1] DBF uptake[b]** (% of control)	**K_i (DBF pH 7.4)** (µM)	**K_i (ES pH 5.5)** (µM)	**Zou et al.[1] K_i (DBF)** (µM)
Acesulfame K	91.7 ± 2.8	94.2 ± 9.8	101 (10 µM)	[a]	[a]	[a]
Advantame	91.5 ± 6.5	91.3 ± 9.9	[a]	[a]	[a]	[a]
Allura Red AC	5.3 ± 1.0	4.4 ± 1.1	3.5 (200 µM)	0.6	1.5	2.59
Aspartame	92.5 ± 7.1	92.0 ± 10.8	103 (200 µM)	[a]	[a]	[a]
Benzoic acid	71.9 ± 4.0	86.0 ± 3.4	[a]	[a]	[a]	[a]
Betanin	129.7 ± 32.9	97.3 ± 8.0	[a]	[a]	[a]	[a]
Brilliant Black BN	115.5 ± 13.2	3.6 ± 2.0	[a]	[a]	3.8	[a]
Brilliant Blue FCF	84.7 ± 6.0	13.1 ± 9.6	19.5 (200 µM)	[a]	17.7	13.0
Carmoisine	33.0 ± 6.9	5.5 ± 1.9	[a]	7.0	2.7	[a]
Curcumin	7.0 ± 0.8	7.4 ± 1.9	[a]	5.0	5.3	[a]
DL - Malic acid	80.3 ± 12.9	101.0 ± 11.4	D-(+) = 96.6 (200 µM) L-(−) = 92.5 (200 µM)	[a]	[a]	[a]
Ethyl paraben	147.7 ± 22.7	88.6 ± 4.1	74.6 (200 µM)	[a]	[a]	[a]
Fumaric acid	141.4 ± 9.7	93.1 ± 4.9	88.9 (200 µM)	[a]	[a]	[a]
Green S	128.9 ± 19.2	83.6 ± 8.4	[a]	[a]	[a]	[a]
Methyl paraben	126.3 ± 8.7	91.9 ± 14.8	92.2 (200 µM)	[a]	[a]	[a]
Neohesperidin DC	37.0 ± 5.4	20.9 ± 0.6	14.7 (200 µM)	35.4	14.9	20.1
Neotame	74.2 ± 9.8	91.3 ± 13.8	67.1 (200 µM)	[a]	[a]	[a]
Rebaudioside A	43.5 ± 2.2	91.5 ± 7.3	[a]	[a]	[a]	[a]
Saccharin	69.9 ± 11.8	82.9 ± 2.7	97.1 (200 µM)	[a]	[a]	[a]
Sodium cyclamate	78.1 ± 15.3	98.0 ± 7.3	[a]	[a]	[a]	[a]
Sorbic acid	77.1 ± 3.1	92.8 ± 7.5	[a]	[a]	[a]	[a]
Stevioside	73.8 ± 5.8	109.1 ± 3.6	[a]	[a]	[a]	[a]
Sucralose	153.5 ± 8.9	90.2 ± 3.5	102 (1000 µM)	[a]	[a]	[a]
Sunset Yellow FCF	71.3 ± 6.3	28.5 ± 10.6	45.5 (50 µM)	[a]	19.6	68.4
Tartrazine	84.9 ± 17.5	80.0 ± 4.6	97.8 (200 µM)	[a]	[a]	[a]

[a] Not determined

[b] Screening concentration in parenthesis

REFERENCES

(1) Zou, L.; Spanogiannopoulos, P.; Pieper, L. M.; Chien, H. C.; Cai, W.; Khuri, N.; Pottel, J.; Vora, B.; Ni, Z.; Tsakalozou, E.; Zhang, W.; Shoichet, B. K.; Giacomini, K. M.; Turnbaugh, P. J. Bacterial Metabolism Rescues the Inhibition of Intestinal Drug Absorption by Food and Drug Additives. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (27), 16009–16018.