

Supplementary material

Chemosphere

Inhibition of human drug transporter activities by succinate dehydrogenase inhibitors

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Table S1. Summary of the experimental conditions for transporter activity assays.

Transporter	Cells	Substrate Method of analysis	Reference inhibitor	Incubation parameters
P-gp	MCF7R	Rhodamine 123 (5.3 μ M) Fluorimetry (485/535 nm)	Cyclosporin A (100 μ M)	Accumulation (30 min)
MRPs	HuH-7	CDCF (3.0 μ M) Fluorimetry (485/535 nm)	Probenecid (2 mM)	Accumulation (30 min) + Efflux (60 min)
BCRP	HEK-BCRP	Hoechst 33342 (16.2 μ M) Fluorimetry (355/460 nm)	Fumitremorgin C (10 μ M)	Accumulation (30 min) + Efflux (90 min)
OATP1B1	HEK- OATP1B1	DCF (10.0 μ M) Fluorimetry (485/535 nm)	BSP (100 μ M)	Accumulation (5 min)
OATP1B3	HEK- OATP1B3	8-FcA (10.0 μ M) Fluorimetry (494/517 nm)	Rifamycin SV (100 μ M)	Accumulation (5 min)
OAT1	HEK-OAT1	6-CF (10.0 μ M) Fluorimetry (485/535 nm)	Probenecid (2 mM)	Accumulation (5 min)
OAT3	HEK-OAT3	6-CF (10.0 μ M) Fluorimetry (485/535 nm) [³ H]-E3S (4.09 nM) Scintillation counting	Probenecid (2 mM)	Accumulation (5 min)
OCT1	HEK-OCT1	ASP ⁺ (10.0 μ M) Fluorimetry (485/607 nm)	Amitriptyline (200 μ M)	Accumulation (5 min)
OCT2	HEK-OCT2	ASP ⁺ (10.0 μ M) Fluorimetry (485/607 nm) [³ H]-Dopamine (13.23 nM) Scintillation counting	Amitriptyline (200 μ M)	Accumulation (5 min)
MATE1	HEK-MATE1	[³ H]-MPP (12.06 nM) Scintillation counting	Amitriptyline (200 μ M)	Accumulation (5 min)
MATE2-K	HEK-MATE2- K	[³ H]-MPP (12.06 nM) Scintillation counting	Amitriptyline (200 μ M)	Accumulation (5 min)

Table S2. Monitored ion transitions for SDHIs and caffeine.

Compounds	Mass to charge (m/z)
Caffein	195.100 \rightarrow 138.10
Benzovindiflupyr	398.000 \rightarrow 342.10
Bixafen	414.000 \rightarrow 394.10
Fluxapyroxad	382.100 \rightarrow 362.10
Pydiflumetofen	425.900 \rightarrow 192.90
Sedaxane	332.100 \rightarrow 159.20

Table S3. Summary of *in vitro* SDHIs effects towards drug transporter activities.

Transporter	SDHIs (10 μ M)														
	Benzovindiflupyr	Bixafen	Boscalid	Carboxin	Fluopyram	Flutolanil	Fluxapyroxad	Furametpyr	Isofetamid	Isopyrazam	Penflufen	Penthiopyrad	Pydiflumetofen	Sedaxane	Thifluzamide
OAT1	↓	=	=	=	=	=	=	=	=	=	=	=	↓	=	=
OAT3	↓↓↓	↓↓↓	↓↓	=	↓	↓↓	↓↓↓	=	↓↓	↓↓	↓↓	↓↓	↓↓↓	↓↓↓	=
OATP1B1	↓↓	↓	=	=	=	=	=	=	=	↓	=	=	=	↓	=
OATP1B3	↓	↓	=	=	=	=	=	=	↓	↓↓	↓	↓↓	↓	=	=
OCT1	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
OCT2	=	=	=	=	=	↓↓	↓↓	=	=	=	↓	=	↓↓↓	↓	=
MATE1	↓	↓	↓	↓	↓	↓	↓	=	↓	↓	↓	↓	↓	↓	↓
MATE2-K	↓	↓	↓	=	↓	↓	↓	=	↓	↓	↓	↓	↓	↓	=
P-gp	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
MRPs	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
BCRP	↓↓↓	↓	=	=	=	=	=	=	=	↓	=	↓	↓	=	=

SDHIs were tested at 10 μ M towards transporter activity in transporter-expressing cells.

=, no change; ↓, transporter inhibition < 50%; ↓↓, 50 % \leq transporter inhibition < 66.6%, ↓↓↓, transporter inhibition \geq 66.6%.

Table S4. List of SDHI molecular descriptors significantly correlated with OAT3 inhibition.

Molecular descriptor		Correlation with OAT3 inhibition %	
Block	Name	Spearman coefficient (r _s)	Significance (p value)
Functional group counts	number of CHR2 (nCHR2)	0.85	0.0004
Geometrical descriptors	quadrupole x-component value / weighted by Sanderson electronegativity (QXXe)	0.76	0.0015
Constitutional indices	sum of atomic van der Waals volumes (scaled on Carbon atom) (Sv)	0.70	0.0048
Geometrical descriptors	quadrupole x-component value / weighted by ionization potential (QXXi)	0.70	0.0048
Geometrical descriptors	quadrupole x-component value / weighted by van der Waals volume (QXXv)	0.68	0.0068
Geometrical descriptors	quadrupole x-component value / weighted by polarizability (QXXp)	0.63	0.0134
Geometrical descriptors	quadrupole x-component value / weighted by I-state (QXXs)	0.63	0.0134
Constitutional indices	number of non-H bonds (nBO)	0.59	0.0224
Functional group counts	number of Pyrazoles (nPyrazoles)	0.60	0.0256
Constitutional indices	percentage of N atoms (N%)	0.55	0.0342
Functional group counts	number of ethers (aliphatic) (nROR)	-0.54	0.0381
Constitutional indices	number of Nitrogen atoms (nN)	0.55	0.0387
Molecular properties	Verhaar Fish base-line toxicity from MLOGP (mmol/l) (BLTF96)	-0.54	0.0408
Molecular properties	Verhaar Algae base-line toxicity from MLOGP (mmol/l) (BLTA96)	-0.54	0.0408
Molecular properties	Moriguchi octanol-water partition coeff. (logP) (MLOGP)	0.54	0.0422
Molecular properties	squared Moriguchi octanol-water partition coeff. (logP ²) (MLOGP2)	0.54	0.0422
Molecular properties	Verhaar Daphnia base-line toxicity from MLOGP (mmol/l) (BLTA96)	-0.54	0.0422

Table S5: Combination index (CI) values for OAT3 inhibition by combined treatment with bixafen and prothioconazole.

% inhibition OAT3 activity	CI ^a	Mixture concentration (μM) ^b	Nature of effect ^c
10	1.20594	0.87	Antagonistic
20	0.73441	1.59	Synergistic
30	0.53987	2.40	Synergistic
40	0.42652	3.38	Synergistic
50	0.34886	4.67	Synergistic
60	0.29001	6.50	Synergistic
70	0.24187	9.45	Synergistic
80	0.19952	15.23	Synergistic
90	0.15830	32.88	Synergistic

^a CI values were calculated using CompuSyn software.

^b The ratio [Bixafen]:[Prothioconazole] is 3:1.

^c Synergistic effect: $\text{CI} < 0.8$; additive effect: $0.8 \leq \text{CI} \leq 1.2$; antagonistic effect: $\text{CI} > 1.2$.

Table S6. Prediction of *in vivo* OCT2 and BCRP inhibition by blood concentrations of SDHIs ^a.

SDHI	ADI (mg/kg/day)	I_{max} ^b	F_u ^c	$I_{\text{max}, u}$ ^d	Transporter	IC_{50}	Ratio $I_{\text{max}, u}/\text{IC}_{50}$	Predicted <i>in vivo</i> inhibition
Pydiflumetofen	0.09	2.95 μM	0.016	0.05 μM	OCT2	2.0 μM	0.025	No inhibition
Benzovindiflupyr	0.05	1.76 μM	0.015	0.03 μM	BCRP ^e	3.9 μM	0.008	No inhibition

^a *In vivo* inhibition of drug transporter activity can be predicted if the ratio $I_{\text{max}, u}/\text{IC}_{50} \geq 0.1$ (Giacomini et al., 2010).

^b defined as the approximated maximum plasma concentration.

^c defined as the unbound plasma fraction and *in silico* predicted using ADMETlab 2.0.

^d defined as the approximated maximum unbound plasma concentration.

^e related to non-intestinal BCRP.

Table S7. Prediction of intestinal BCRP inhibition by benzovindiflupyr ^a.

SDHI	ADI (mg/kg/day)	I_{gut} ^b	Transporter	IC_{50}	Ratio $I_{\text{gut}}/\text{IC}_{50}$	Predicted <i>in vivo</i> inhibition
Benzovindiflupyr	0.05	35.2 μM	BCRP	3.9 μM	9.0	No inhibition

^a *In vivo* inhibition of intestinal BCRP activity can be predicted if the ratio $I_{\text{gut}}/\text{IC}_{50} \geq 10$ (Giacomini et al, 2010).

^b defined as the luminal gut concentration.

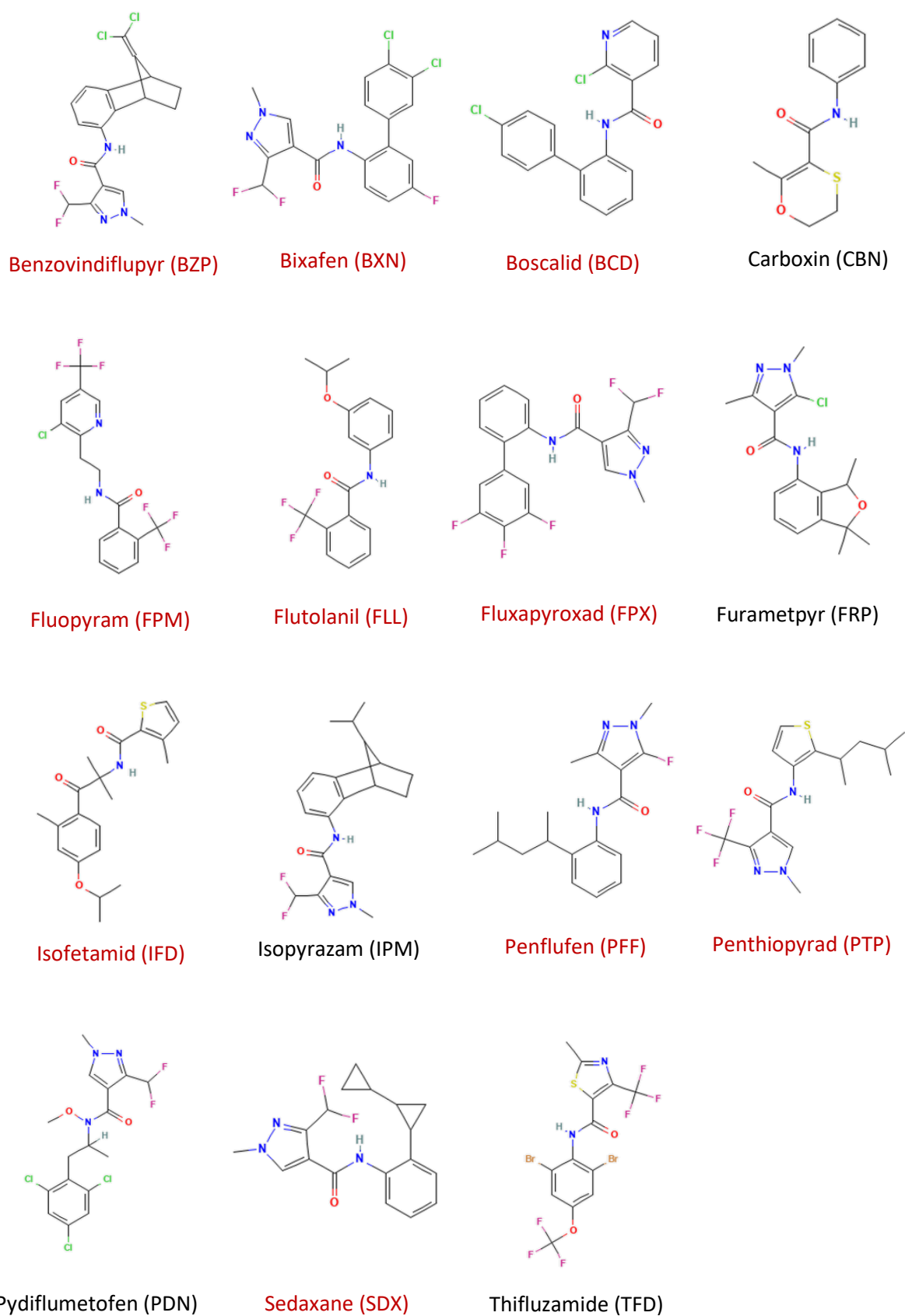


Fig. S1. Chemical structures of SDHIs. SDHIs currently authorised in the European Union are indicated in red.

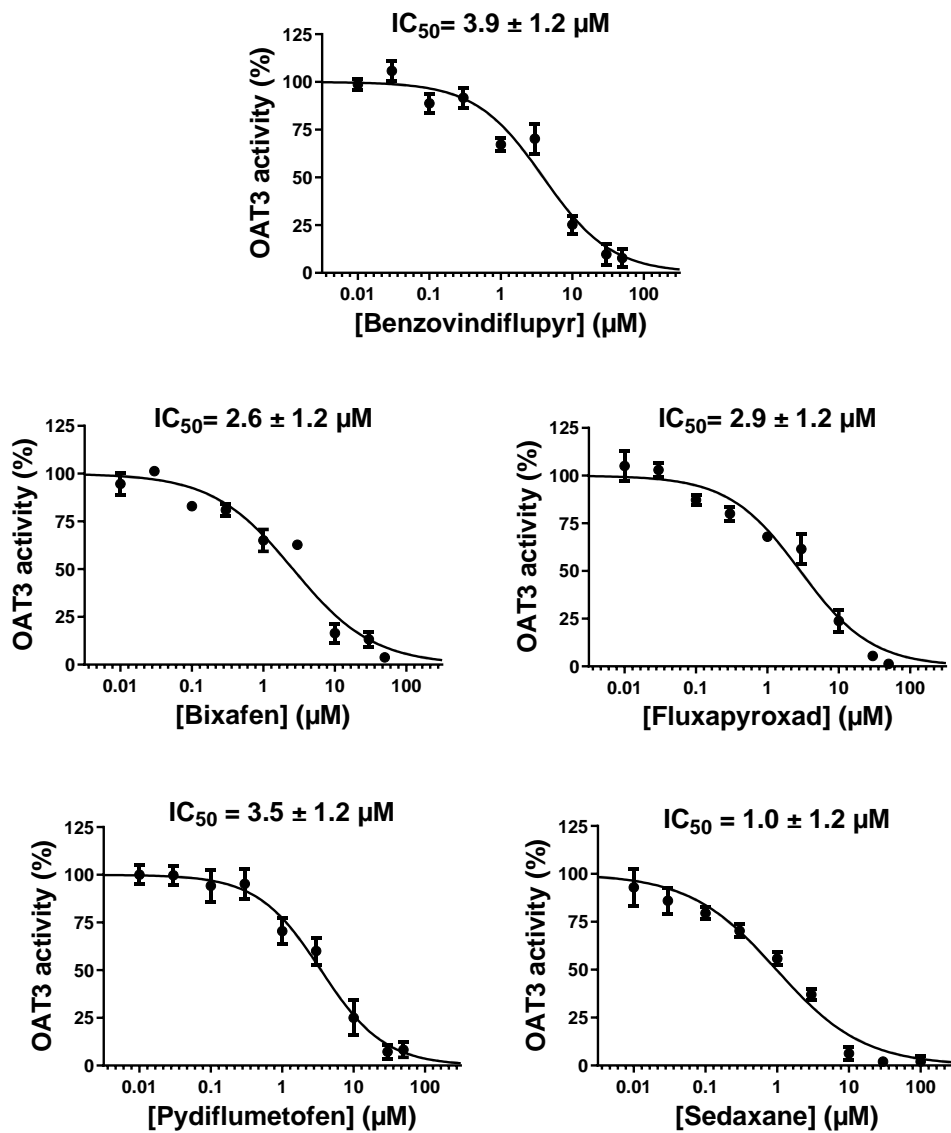


Fig. S2. Concentrations-dependent effects of SDHIs towards OAT3 activity. Various concentrations of benzovindiflupyr, bixafen, fluxapyroxad, pydiflumetofen and sedaxane were tested against OAT3-mediated uptake of the fluorescent dye 6-CF in HEK-OAT3 cells. Data are expressed as % of transporter activity found in control cells not exposed to SDHIs, arbitrarily set at 100 %. They are the means \pm SEM of at least three independent experiments. The IC_{50} values are indicated at the top of the graphs.

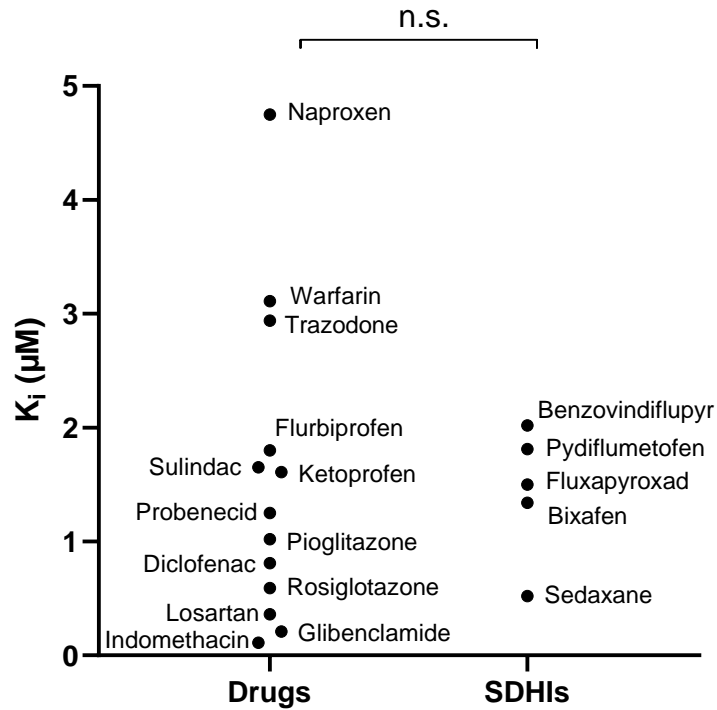


Fig. S3. Comparison of SDHI and drug K_i values for inhibition of OAT3-mediated transport of 6-CF. K_i values for drugs ($n=12$) inhibiting OAT3 with high affinity are from Zou et al., 2021. n.s., not statistically significant.

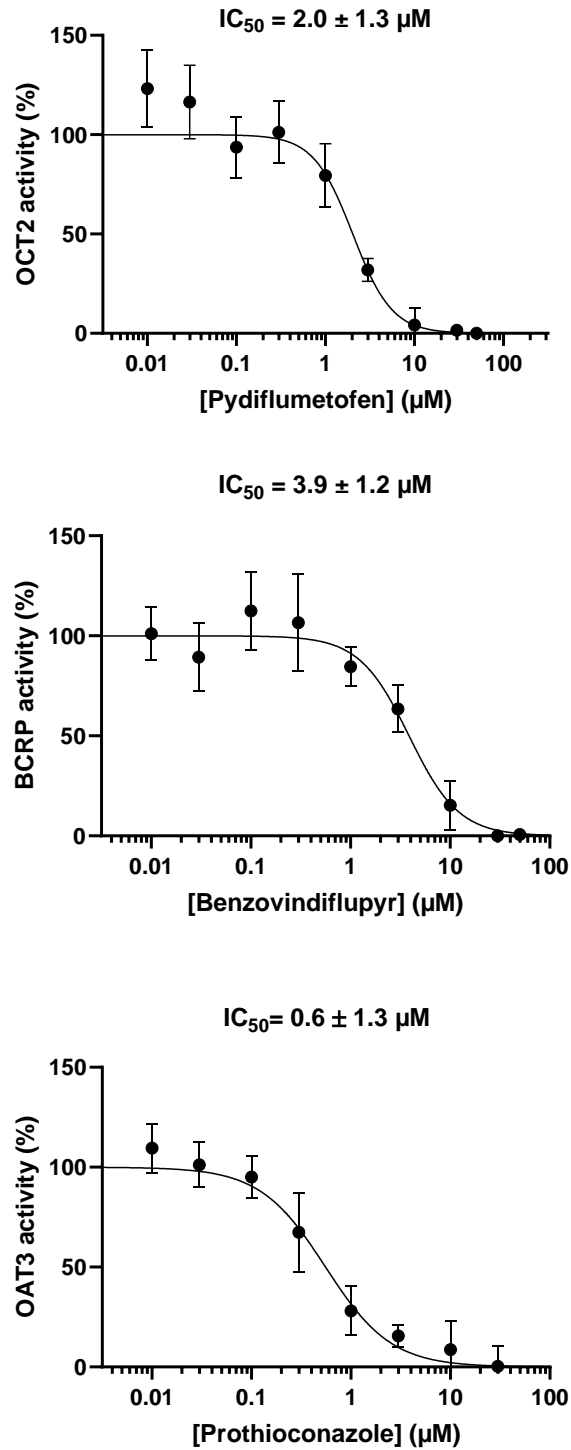
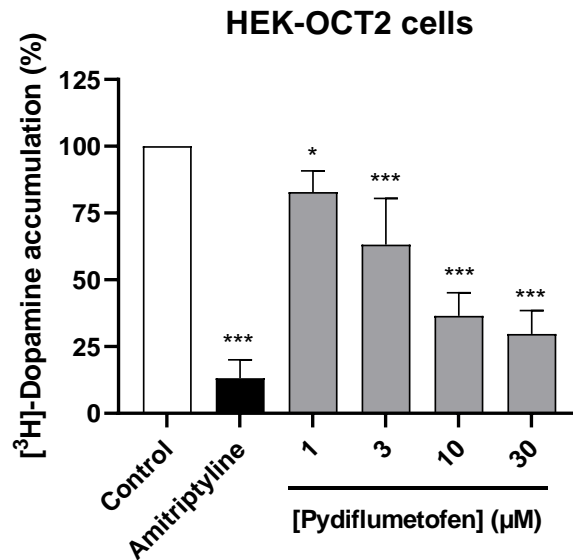


Fig. S4. Concentrations-dependent effects of pydiflumetofen, benzovindiflupyr and prothioconazole towards OCT2, BCRP and OAT3 activities. Data are expressed as % of the transporter activity in control cells not exposed to fungicides, arbitrarily set at 100%. They are the means \pm SEM of at least three independent assays. IC_{50} values are indicated at the top of the graphs.



*Fig. S5. Effects of pydiflumetofen towards OCT2-mediated transport of the endogenous substrate dopamine. HEK-OCT2 cells were incubated with 13.23 nM [³H]-dopamine for 5 min at 37°C in the absence (control) or presence of various concentrations of pydiflumetofen or of the reference OCT2 inhibitor amitriptyline. Intracellular accumulation of dopamine was next determined by scintillation counting. Data are expressed as % of dopamine accumulation in control cells, arbitrarily set at 100%, and are the means ± SEM of at least three independent experiments. *, $p < 0.05$ and ***, $p < 0.001$ when compared to dopamine accumulation in control cells.*

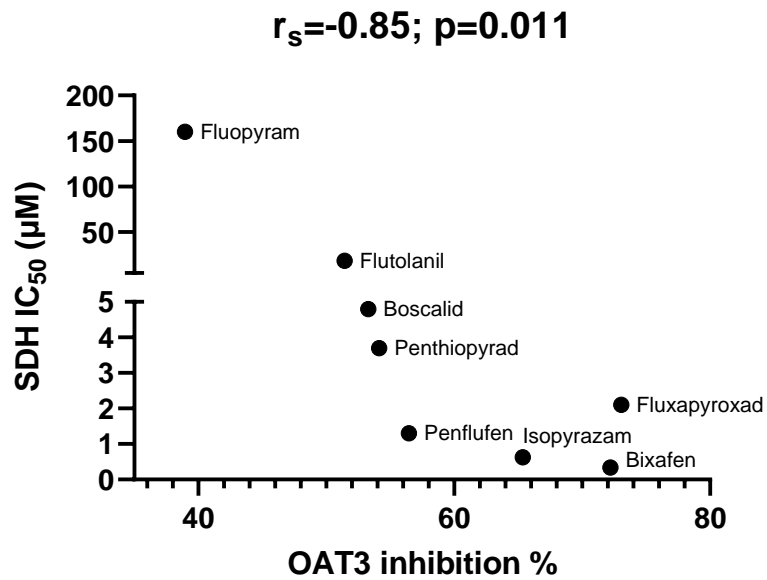


Fig. S6. Spearman rank correlation analysis between the percentages of OAT3 inhibition and the SDH IC₅₀ values for 8 SDHIs. SDHI IC₅₀ values for SDH inhibition are from Benit et al., 2019, and the OAT3 inhibition percentages from Fig. 2. The Spearman rank coefficient (r_s) and the p value are indicated at the top of the graph.

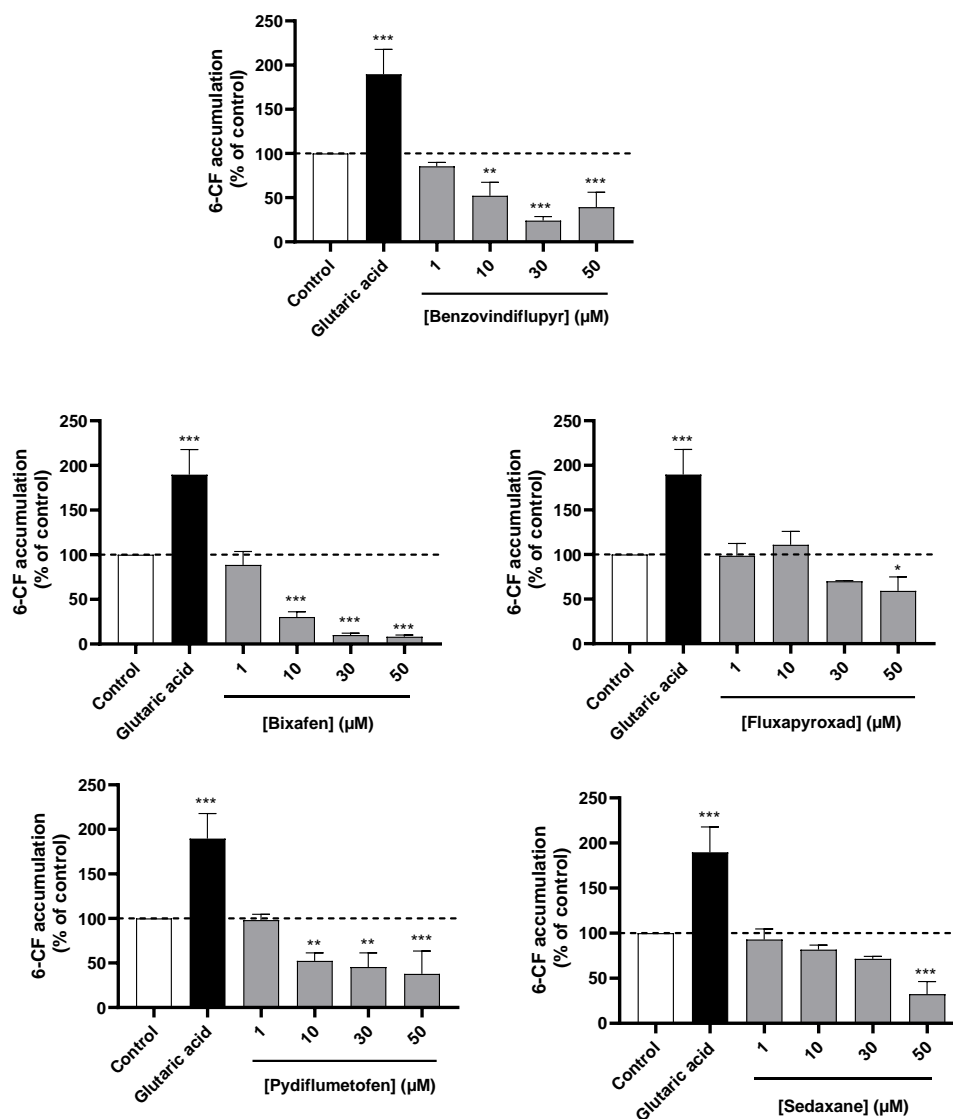


Fig. S7. Lack of trans-stimulation of OAT3 activity by SDHIs. HEK-OAT3 cells were first incubated in the absence (control) or presence of various concentrations of SDHIs or 1 mM glutaric acid (used here as a positive trans-stimulating agent) for 15 min at 37 °C. After washing, cells were next incubated with 10 μM 6-CF for 5 min at 37 °C. Accumulation of 6-CF was finally determined by spectrofluorimetry. Data are expressed as % of 6-CF accumulation in control cells, arbitrarily set at 100 % and indicated by dashed lines on the graphs. They are the means \pm SEM of at least three independent experiments. *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$ when compared to control.