Title: Interactions of retinoids with the ABC transporters P-glycoprotein and Breast Cancer Resistance Protein

Running title: Interactions of retinoids with ABCB1 and ABCG2

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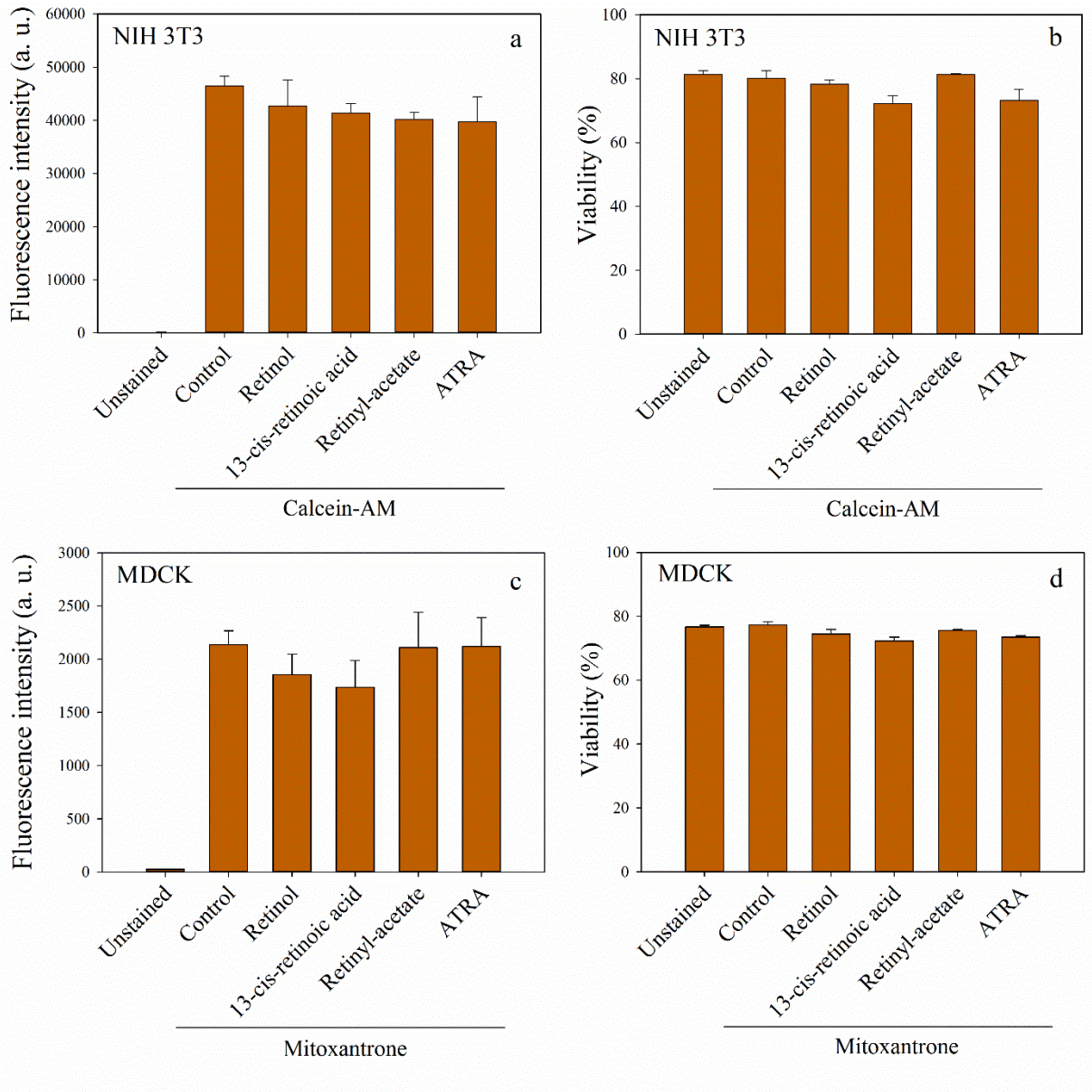
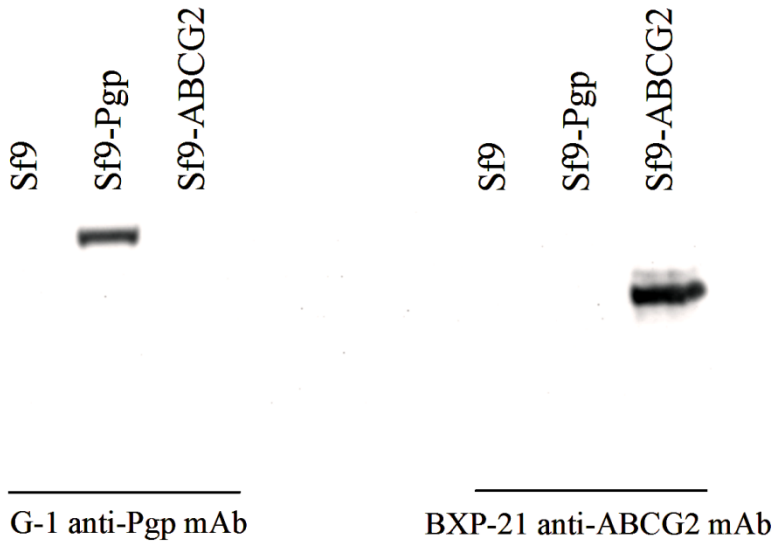
Keywords: retinoid derivatives, P-glycoprotein, ABCG2, ABC transporter, membrane fluidity, intramembrane localization

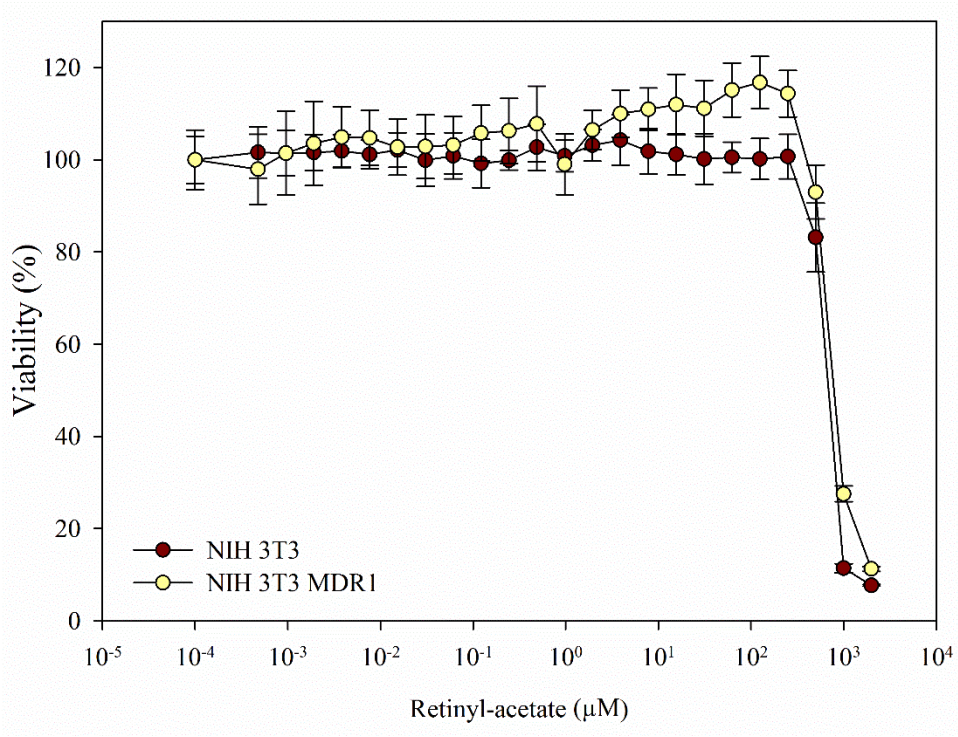
SUPPLEMENTARY FIGURES



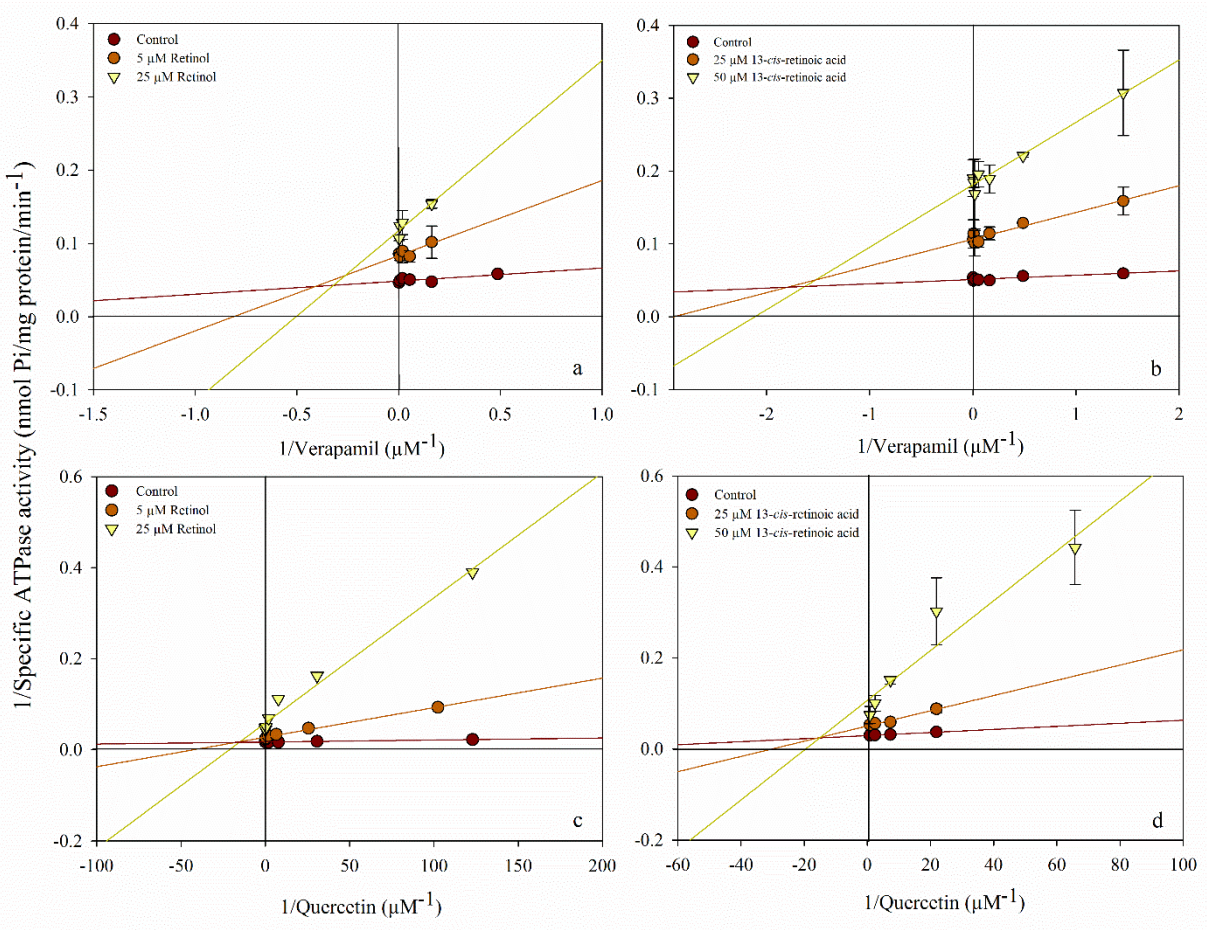
Supplementary Fig. S1: Effect of retinoid derivatives on the calcein (a) and mitoxantrone (c) accumulation and viability (b, d) of Pgp- and ABCG2-negative NIH 3T3 and MDCK cells. Cells were pre-treated with retinoids added at 50 μM concentration for 20 min at 37°C and then the NIH 3T3 cells were stained with 500 nM calcein-AM for 20 min (a), while the MDCK cells (b) were stained with 5 μM mitoxantrone for 40 min at 37°C. Cell viability was determined on the basis of propidium iodide exclusion.



Supplementary Fig. S2: Western blot of membrane samples prepared from Sf9 cells expressing wild-type Pgp and ABCG2. Non-transfected Sf9 membrane sample is shown as a control. Preparation of membrane vesicles was carried out as described in Materials and Methods. The immunoblot was developed by the human Pgp-specific mouse monoclonal antibody G-1 and ABCG2-specific antibody BXP-21 (see Materials and Methods).



Supplementary Fig. S3: Cytotoxic effect of retinyl-acetate on Pgp-positive and Pgp-negative NIH 3T3 cells. The AlamarBlue (Serotec, UK) based *in vitro* cytotoxicity assay was performed as previously described [1]. Cells were seeded in 96-well plates at a cell density of 5 × 10^3 cells/well. 24 hours later retinyl-acetate was added at different concentrations and the plates were further incubated for 72 h at 37 °C. The cell viability was determined measuring the 530/590 nm fluorescence intensity of the dye in an automated microplate reader (BioTec Synergy HT, US). The AlamarBlue fluorescence intensity of the samples was normalized to the fluorescence of the retinyl-acetate untreated samples, and plotted as a function of retinyl-acetate concentration. The data points are means of eight parallel samples (±SD).



Supplementary Fig. S4: Lineweaver-Burk re-plots of the inhibition of the verapamil-stimulated ATPase activity of Pgp (a,b) and the quercetin-stimulated ATPase activity of ABCG2 (c,d) by retinol and 13-*cis*-retinoic acid. Verapamil and quercetin were applied at 50 µM and 10 µM concentrations, respectively. Representative data are shown out of three independent experiments (data points are means ± SD, n=3). The X-intercept of a Lineweaver-Burk plot is $-1/K_m$, while the Y intercept is $1/v_{max}$. Thus, retinoid treatments decrease v_{max} and increase K_m indicative of mixed-type inhibition (i.e. retinoids interfere with substrate binding *and* reduce the effectiveness of turnover).

SUPPLEMENTARY TABLES

Drug	IC_{50} values (μM)			
	Pgp		**ABCG2**	
	Basal activity	Stimulated activity	Basal activity	Stimulated activity
Retinol	23.5±4	27.9±0.7	31.6±4.2	95.2±10.6
All-*trans*-retinoic-acid	No effect	No effect	No effect	No effect
9-*cis*-retinoic acid	No effect	No effect	No effect	No effect
13-*cis*-retinoic acid	60.5±11.4	26.8±2	70.6±9	76.5±2.3
All-*trans*-4-oxo-retinoic acid	No effect	Weak inhibition at high conc.	No effect	No effect
Retinyl-acetate	36.8±4	19.1±5	18.9±4	70.4±7.3
Retinyl-propionate	No effect	No effect	No effect	No effect
Retiny-palmitate	No effect	No effect	No effect	No effect

Supplementary Table S1. Effects of retinoid derivatives on the ATPase activity of Pgp and ABCG2. The IC_{50} values were calculated by fitting the dose-response curves with a four parameter logistic function ($R^2 \geq 0.98$, SigmaPlot 12.0).

Drug	LogP	Reference
Quercetin	1.8	[2]
Cyclosporin A	2.9	[3]
Ko143	3.5	CID 449171
Retinol	5.7	CID 445354
All-*trans*-retinoic-acid	6.3	CID 444795
9-*cis*-retinoic acid	6.3	CID 449171
13-*cis*-retinoic acid	6.3	CID 5282379
All-*trans*-4-oxo-retinoic acid	n. d.	n. d.
Retinyl-acetate	6.3	CID 638034
Retinyl-propionate	6.7	CID 6394572
Retiny-palmitate	13.6	CID 5280531

Supplementary Table S2. Octanol-water partition coefficients ($LogP_{ow}$ values) of the studied compounds. The values were collected from the National Center for Biotechnology Information. PubChem Compound Database Identification (CID) values are listed in the table.

PUBCHEM REFERENCES:

Ko143: National Center for Biotechnology Information. PubChem Compound Database; CID=10322450, https://pubchem.ncbi.nlm.nih.gov/compound/10322450 (Assessed Oct. 25, 2006)

Retinol: National Center for Biotechnology Information. PubChem Compound Database; CID=445354, https://pubchem.ncbi.nlm.nih.gov/compound/445354 (Assessed Sep. 16, 2004)

ATRA: National Center for Biotechnology Information. PubChem Compound Database; CID=444795, http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=444795 (Assessed Sep. 16, 2004)

9-*cis*-retinoic acid: National Center for Biotechnology Information. PubChem Compound Database; CID=449171, http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=449171 (Assessed Mar. 25, 2005)

13-*cis*-retinoic acid: National Center for Biotechnology Information. PubChem Compound Database; CID=5282379, http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5282379 (Assessed Jun. 24, 2005)

Retinyl-acetate: National Center for Biotechnology Information. PubChem Compound Database; CID=638034, http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=638034 (Assessed Mar. 27, 2005)

Retinyl-palmitate: National Center for Biotechnology Information. PubChem Compound Database; CID=5280531, http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5280531 (Assessed Sep. 16, 2004)

Retinyl-propionate: National Center for Biotechnology Information. PubChem Compound Database; CID=6394572, http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6394572 (Assessed Oct. 07, 2005)

REFERENCES FOR SUPPLEMENTARY INFORMATION

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