Supplemental Data

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Nelfinavir and its active metabolite M8 are partial agonists and competitive antagonists of the human pregnane X receptor

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Supplemental Materials and Methods

Cell viability

HepG2 or H-P cells were seeded into white flat-bottom CELLSTAR® 96-well plates with µClear® bottom (Greiner Bio-One, Frickenhausen, Germany), with 40,000 cells per well in a volume of 100 µl and incubated for 24 hours. Then, cells were treated for further 24 hours with varying doses of nelfinavir or metabolite M8, ranging from 1 to 50 µM, or vehicle only (0.5% DMSO). Each treatment was performed in technical triplicates. Afterwards, cell viability was determined based on ATP content, using the CellTiter-Glo® luminescent cell viability assay (Promega), as specified by the manufacturer. Luminescence was measured with the 2300 EnSpire multimode plate reader (Perkin Elmer, Rodgau, Germany).

Supplemental Table S1 Hepatocyte donor data

ID	ethnicity	sex	age cohort	diagnosis	long-term medication	used in
GH41	European	female	30-39	liver metastasis CRC	n.s.	Fig. 7A
GH42	European	female	50-59	focal nodular hyperplasia	none	Fig. 7A
GH43	European	female	30-39	focal nodular hyperplasia	n.s.	Fig. 7A
GH44	European	male	50-59	liver metastasis CRC	vitamin D	Fig. 7A
GH45	European	female	60-69	liver metastasis CRC	pantoprazole	Fig. 7A
GH46	n.s.	Male	30-39	liver metastasis CRC	pantoprazole	Fig. 7A
GH61	n.s.	female	70-79	liver metastasis breast carcinoma	ramipril, L-thyroxine	Fig. 7B
GH62	European	Male	70-79	liver metastasis CRC	acetylsalicylic acid, bisoprolol, simvastatin, ticagrelor	Fig. 7B
GH63	European	Female	30-39	liver metastasis CRC	tinzaparin	Fig. 7B
GH64	European	Male	60-69	intrahepatic CCC	salbutamol, unspecified hypertensive drug(s), unspecified ACE inhibitor(s)	Fig. 7B

n.s., not specified; CRC, colorectal carcinoma; CCC, cholangiocellular carcinoma

Supplemental Table S2. Overview of binding site characteristics and docking scores.

PDB ID	Pocket	Size	PLB	Docking Scores	
				NFV	M8
1M13	LBP	245	4.6	101.4	104.3
C284 A	AF-2	n.d.	n.d.	77.07	76.2
	Alt-1	60	1.4	82.9	80.3
	Alt-2	56	0.8	68.4	71
1M13	LBP	244	4.6	104.4	106.1
C284 B	AF-2	n.d.	n.d.	75.3	74.8
	Alt-1	60	1.48	78.1	80.7
	Alt 2	64	0.91	66.7	67.8
1NRL	LBP	190	4.4	107.4	110.4
Chain A	AF-2	n.d.	n.d.	76.8	78
	Alt-1	32	1.37	64.7	67.4
	Alt-2	45	1.16	71	66.8
1NRL	LBP	190	3.8	97	100
Chain B	AF-2	n.d.	n.d.	81.1	78.2
	Alt-1	67	1.9	63.7	68.2
	Alt-2	41	1.2	76.3	78.3
2O9I	LBP	186	4	104	103.2
Chain A	AF-2	n.d.	n.d.	75.3	78.5
	Alt-1	52	1.8	73.4	72.1
	Alt-2	46	1	73.9	75.1
2O9I	LBP	186	4	98.4	101
Chain B	AF-2	n.d.	n.d.	84.2	85.4
	Alt-1	52	1.8	65.2	63.7
	Alt-2	46	1	70.3	79.3

For the PXR LBD structures used in this study, the calculated size (number of alpha spheres) of the LBP, AF-2 groove, and the alternative pockets (Alt-1, Alt-2) are listed, along with the calculated propensity for ligand binding (PLB) at these sites. For each pocket, the top-ranked docking scores of nelfinavir (NFV) and M8 are provided. n.d., not determined.

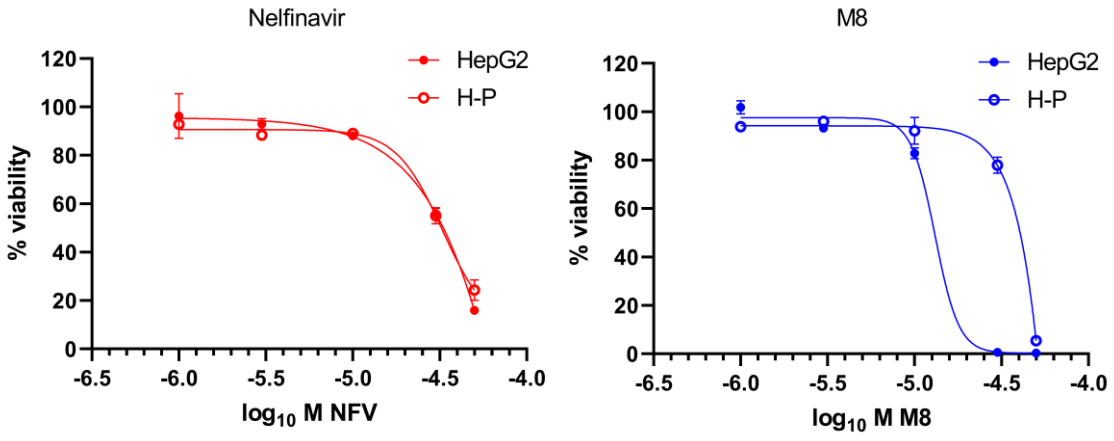
Supplemental Table S3. Number of docking poses per cluster and their ranking according to the docking score within the set of 100 docking conformations

Cluster	Nelfinavir		M8	
	Cluster Size	Docking Poses	Cluster Size	Docking Poses
1	8	1,2,4,5,6,8,16,51	4	1,2,5,11
2	1	3	4	3,4,24,27
3	4	7,9,13,14	3	6,12,42
4	1	10	4	7,9,21,32
			3	8,16,73
			1	10

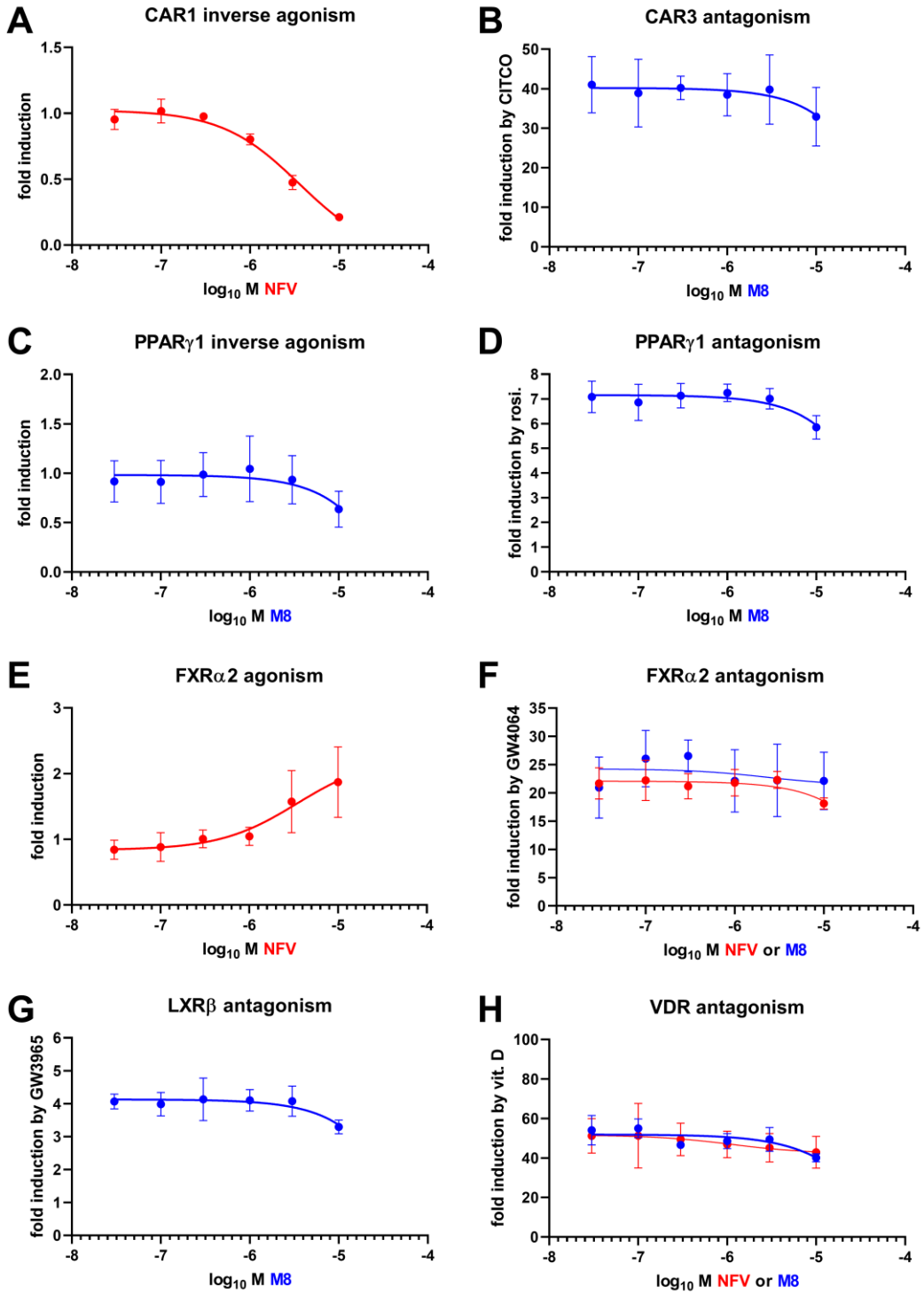
Supplemental Table S4 Rifampin EC_{50} with increasing doses of nelfinavir and M8

NFV	Rifampin		M8	Rifampin	
[µM]	EC_{50} [µM]	95% CI [µM]	[µM]	EC_{50} [µM]	95% CI [µM]
0	2.0	1.2 - 3.3	0	1.6	0.97 - 2.7
3	5.4	3.4 – 8.8	3	2.1	1.1 - 4.1
10	19.7	6.9 - 158	10	5.9	5.4 - ???

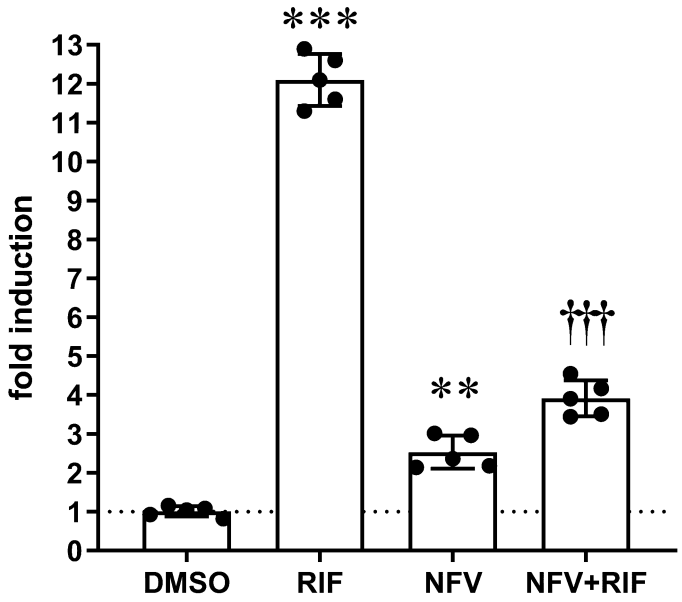
???, not computable by GraphPad Prism



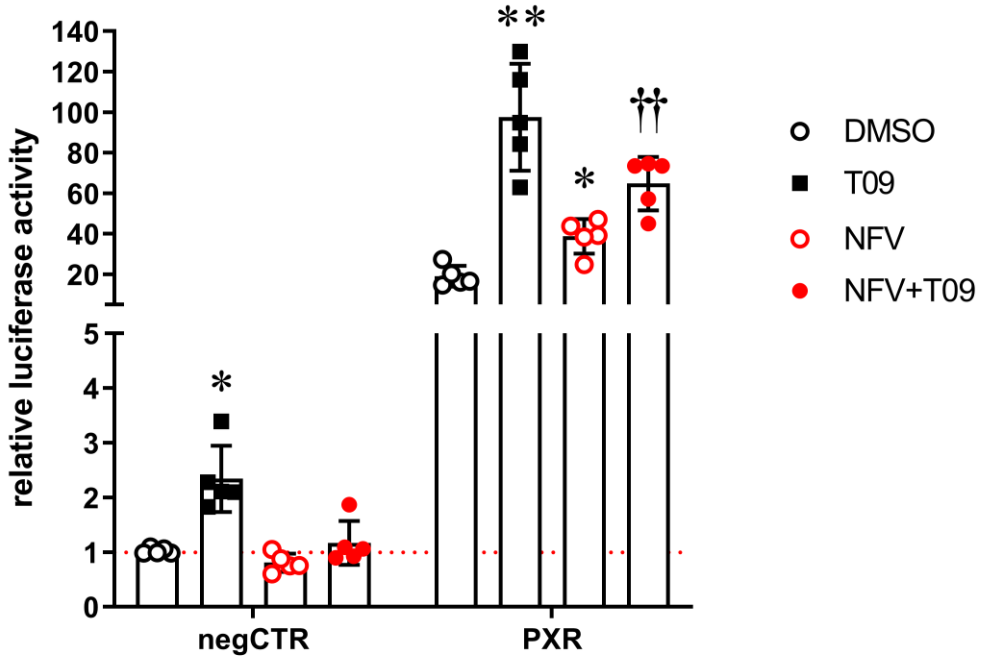
Supplemental Fig. S1. Cell toxicity of nelfinavir and M8. HepG2 and H-P cells were treated with increasing concentrations of nelfinavir or M8 metabolite for 24 h. Means ± SD (*N*=2) are shown.



Supplemental Fig. S2. Concentration response analyses of the effects of nelfinavir and/or M8 on nuclear receptors beside PXR. HepG2 cells were co-transfected with expression plasmids encoding human CAR1 (A), CAR3 (B), PPARγ1 (C, D), FXRα2 (E, F), LXRβ (G) or VDR (H) and the corresponding reporter gene plasmids (see Materials and Methods). Transfected cells were treated with increasing concentrations of nelfinavir (NFV) or M8, alone (A, C, E) or in combination with respective receptor agonists (B, D, F-H), as indicated. Mean fold induction ± SD (*n*=3) by the respective treatment is shown, with respect to the normalized reporter activity of cells treated with vehicle DMSO only, which was designated as 1. Non-linear fit of dose response was executed as described in Materials and Methods. rosi., rosiglitazone; vit. D, 1α,25-dihydroxyvitamin D3.



Supplemental Fig. S3. Effect of nelfinavir on the GAL4-PXR-LBD fusion protein. HepG2 cells were transfected with expression plasmid encoding GAL4-DBD/PXR-LBD(108-434) fusion protein. Transfected cells were treated with 0.1% DMSO or 10 µM nelfinavir (NFV) in the absence or presence of 10 µM rifampin (RIF) for 24h. Data are presented as scatter plots with means (columns) ± S.D. (*n*=5) of normalized luciferase activity of co-transfected pGL3-G5, relative to the activity of cells treated with vehicle DMSO only. Differences to respective treatments with DMSO (asterisks, exclusively for single compound treatments) or rifampin alone (daggers, exclusively for rifampin co-treatment) were analyzed by repeated measures one-way ANOVA with Dunnett's multiple comparisons test or paired t-test, respectively.



Supplemental Fig. S4. Effect of nelfinavir on PXR activation by the high affinity agonist T0901317. HepG2 cells were transfected with empty vector pcDNA3 (negCTR) or expression plasmid encoding human PXR and treated with 0.1% DMSO or 10 µM nelfinavir (NFV), with or without 1 µM T0901317 (T09) for 24 h. Data are presented in scatter plots with means (columns) ± SD (n=5) of normalized luciferase activity of co-transfected CYP3A4 reporter, relative to the activity of cells transfected with pcDNA3 and treated with DMSO only. Differences to respective treatments with DMSO (asterisks, exclusively for single compound treatments) or T0901317 alone (daggers, exclusively for T0901317 co-treatments) were analyzed by repeated measures two-way ANOVA with Dunnett's multiple comparisons test. *, P<0.05; **, †† P<0.01.