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HIV protease inhibitors interact with ABCC4/MRP4: a basis for unanticipated enhanced cytotoxicity

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Supplementary data

Methods

ATPase activity measurement

The assay measured the amount of P_i released over 20 min at 37 °C in the ATPase assay buffer (50 mM MES-Tris, pH 6.8, 50 mM KCl, 5 mM sodium azide, 2 mM EGTA, 2 mM dithiothreitol, and 10 mM $MgCl_2$). The assay was carried out under basal conditions or in the presence of putative substrates or modulators. The reaction was initiated with 5 mM ATP and quenched with SDS (2.5% final concentration). The amount of P_i released was measured using a colorimetric assay.

PMEA efflux in the presence of NFV

Wild-type or ABCC4/Mrp4 knockout MEFs were washed and incubated in ATP-depleting medium (DMEM without glucose, L-glutamine, sodium pyruvate (Cellgro, Manassas, VA) supplemented with 10 mM sodium azide and 10 mM 2-deoxy-glucose) for 30 min. Medium was then removed, and cells were incubated

with fresh ATP-depleting medium containing 10 μM Bis(POM)-PMEA (with a trace amount of [^{3}H]Bis(POM)-PMEA) for 30 min to allow intracellular accumulation of PMEA. The cells were then washed twice with cold PBS and incubated in warm transport medium for the indicated duration in the absence or presence of 50 μM NFV. PMEA efflux was measured by monitoring the amount of radioactivity both in the media and in the cells by scintillation counting.

Bis(POM)-PMEA and RTV uptake and proliferation assay

Saos2 or Hek293 cells containing either vector alone or MRP4 were incubated with Bis(POM)-PMEA (with a trace amount of [^{3}H]Bis(POM)-PMEA) at a final concentration of 10 μM or with RTV (with a trace amount of [^{3}H]-RTV) at 0.5 μM or 2 μM for 3 hr. The medium was removed, and the cells washed extensively with cold PBS. Cells were then lysed with 0.5 M NaOH, and the amount of radioactivity in the lysate was measured by scintillation counting. Intracellular PMEA levels were normalized to the protein concentration in the lysate as measured by Bradford assay.

Cell proliferation assay was performed as described in the main text. In brief, Hek293 cells were cultured overnight in a 96 well plate, treated with various concentrations of Bis(POM)-PMEA in the presence or absence of 60 μM RTV for 6 hr, followed by an incubation in drug-free MTT medium for 3 days.

Supplementary Table S1. Input details for pharmacophore construction

Compound	MaxOmitFeat	Principal
Ritonavir	0	1
Nelfinavir	0	2
Amprenavir	0	0
Indinavir	0	0
Saquinavir	0	0

Supplementary Table S2. Training set for diverse MRP4 models

Compound	Activ	Principal
Dipyridamole	1	2
Quercetin	1	2
MK-571	2	1
Indomethacin	5	1
Sulindac	2	1
Losartan	10	1
Sildenafil	20	0
AEBSF	750	0
Celecoxib	35	0
Probenecid	100	0

Supplementary Table S3. MRP4 model 1

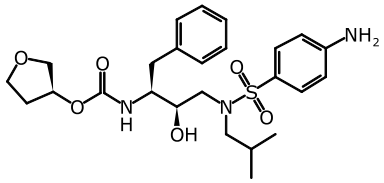
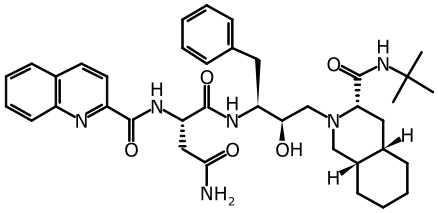
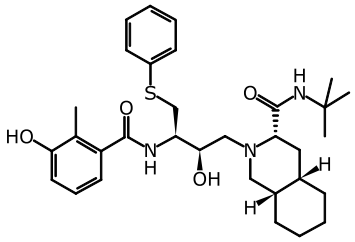
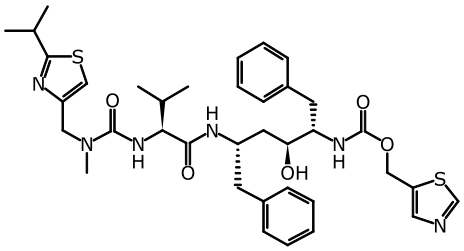
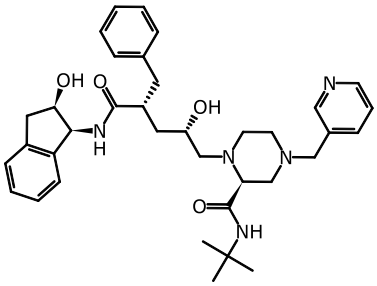
FitValue	TestSubstance_ChemicalName
2.34022	Methyl hesperidin
2.05303	Nelfinavir mesylate
1.81393	Digitoxin
1.57506	Hesperidin
1.08746	Chromomycin A3
0.894553	Methyldigoxin
0.758604	Pepstatin
0.49864	Echinacoside
0.368538	Lanatoside A
0.254237	Vancomycin hydrochloride
0.200317	Deslanoside
0.127627	Lanatoside B

Supplementary Table S4. MRP4 model 2

FitValue	TestSubstance_ChemicalName
7.99848	Nelfinavir mesylate
4.52654	Atazanavir
2.79973	Ioxaglic acid
2.50273	Mepartricin
2.4375	Deslanoside
2.42177	Nicofuranose
1.80846	Bisibutiamine
1.75545	Virginiamycin S1
1.50905	Triptorelin pamoate
1.30721	Leucomycin tartrate
0.876732	Josamycin

Supplementary figure S1

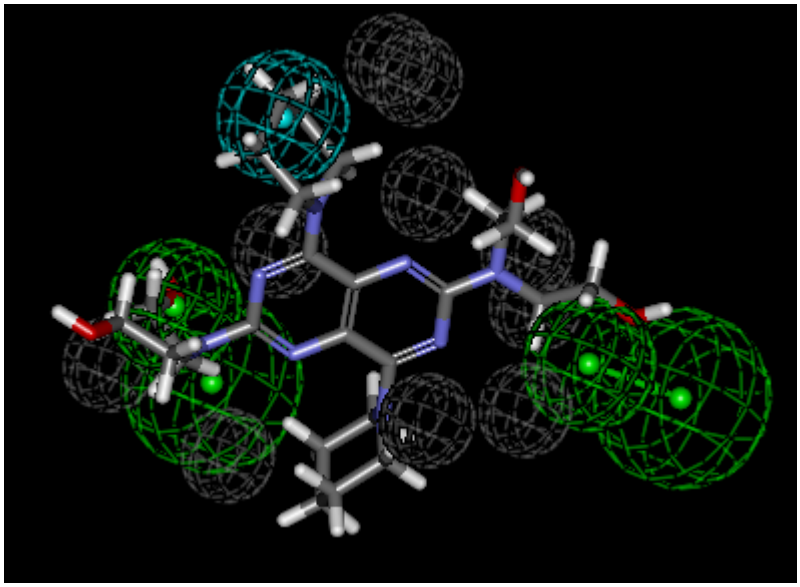
Structures of HIV protease inhibitors

Molecule	Name	MWT
	Amprenavir (APV)	505.64
	Saquinavir (SQV)	670.90
	Nelfinavir (NFV)	663.90
	Ritonavir (RTV)	720.96
	Indinavir (IDV)	613.81

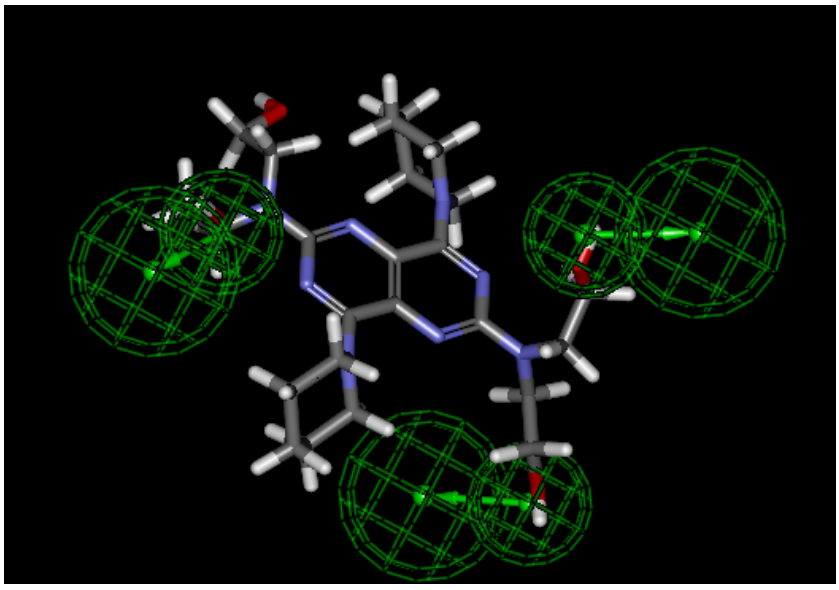
S1 Structures of HIV protease inhibitors used in the study.

Supplementary figure S2

A

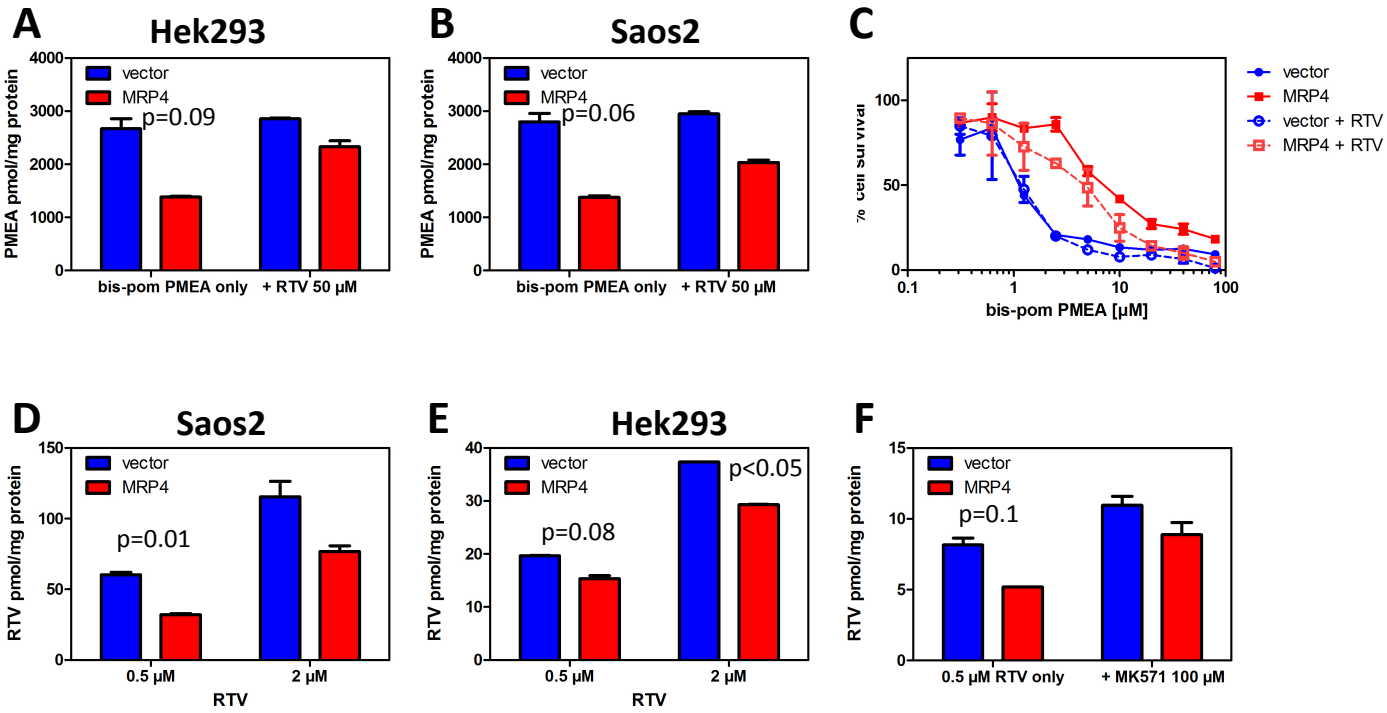


B



S2 Inhibitor pharmacophores for MRP4. (A) dipyridamole mapped to common features pharmacophore – green = hydrogen bond acceptor, cyan = hydrophobic, grey = excluded volumes (space to avoid). (B) Dipyridamole mapped to quantitative pharmacophore.

Supplementary figure S3



S3 Ritonavir is a weak MRP4 inhibitor and substrate. Bis-(POM) PMEA uptake was determined in (A) Hek293 or (B) Saos2 cells overexpressing either vector alone or MRP4 in the presence or absence of 50 μM RTV. (C) Presence of 60 μM RTV partially sensitized Hek293 cells overexpressing MRP4 to Bis-(POM) PMEA cytotoxicity. Uptake of radiolabeled RTV was reduced in (D) Saos2 or (E) Hek293 cells overexpressing MRP4. (F) MRP4 inhibitor, MK571, increases RTV accumulation in Hek293 cells overexpressing MRP4.