

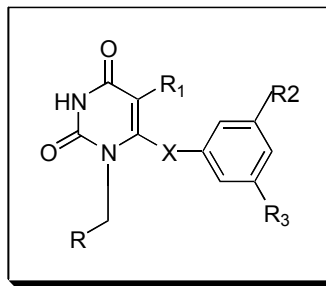
Development of Pyrimidinedione NNRTIs With a High Genetic Barrier to Resistance

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Antiviral Profile of the Pyrimidinediones

- Highly potent inhibitors of HIV-1 with activity at sub-nanomolar concentration levels and TI values greater than one million.
- Slight (~10-100-fold) loss of efficacy against NNRTI-resistant HIV-1.
- Active against HIV-2 at nanomolar concentration levels with TIs > 100,000.
- Inhibits HIV-1 reverse transcriptase; no activity versus HIV-2 reverse transcriptase.
- Active in virus entry inhibition, fusion inhibition, and cell-to-cell transmission inhibition assays.
- *Pyrimidinediones represent a new class of NNRTI with an intrinsically high barrier to resistance due to their dual mechanism of action.*

Highly Active Lead Molecules from SAR Evaluations

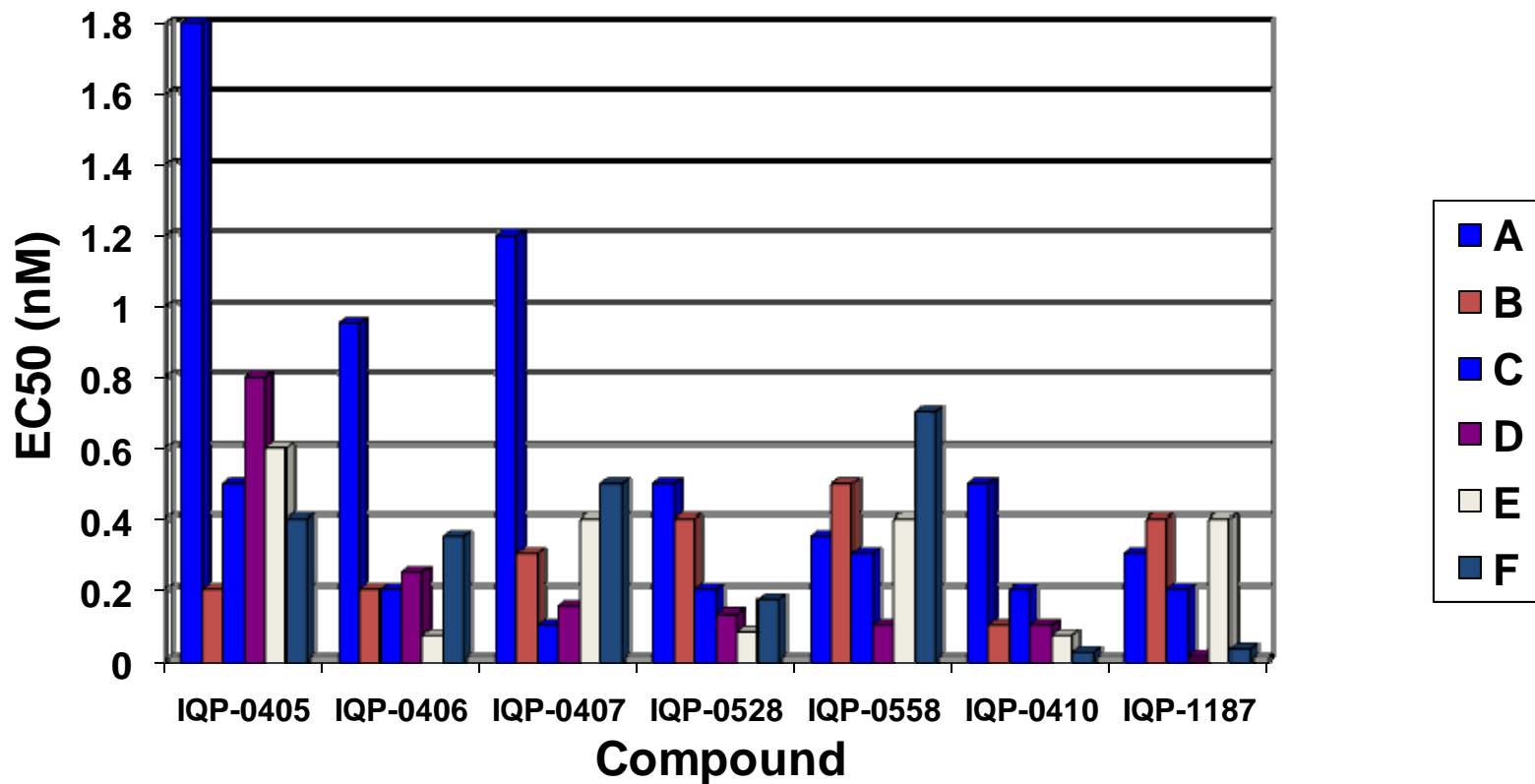


Compound	Substituents				
	R1	R2	R3	X	R
IQP-0405	Et	Me	Me	O	Cyclopropyl
IQP-0406	iPr	Me	Me	O	Cyclopropyl
IQP-0407	Et	Me	Me	C=O	Cyclopropyl
IQP-0528	iPr	Me	Me	C=O	Cyclopropyl
IQP-0558	iPr	Me	Me	C=O	1-Cyclopenten-1-yl
IQP-0410	Et	Me	Me	C=O	3-Cyclopenten-1-yl
IQP-1187	iPr	Me	Me	C=O	3-Cyclopenten-1-yl

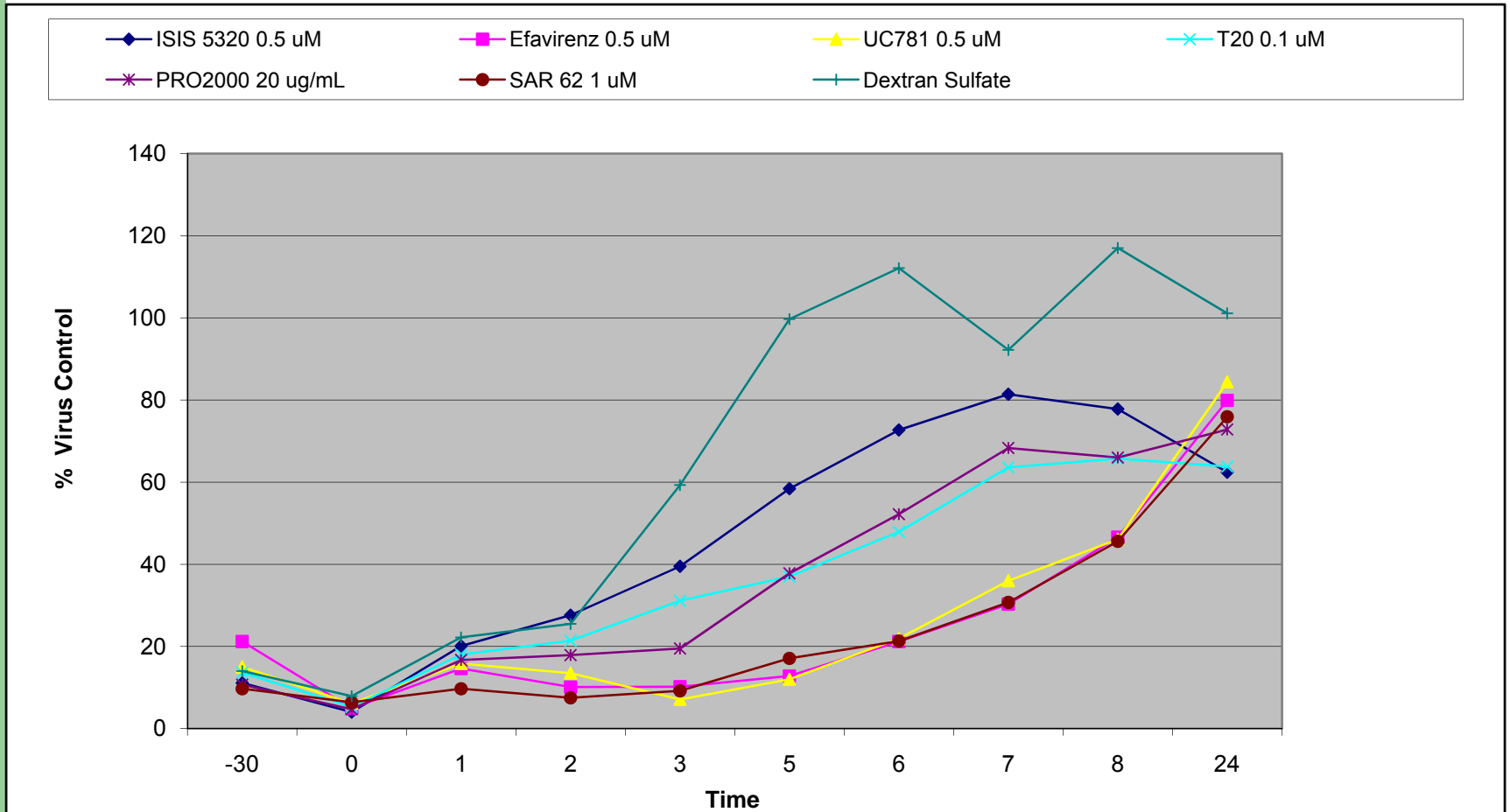
IQP-0410: lead therapeutic candidate

IQP-0528: lead microbicide candidate

Inhibition of Clinical HIV-1 Strains

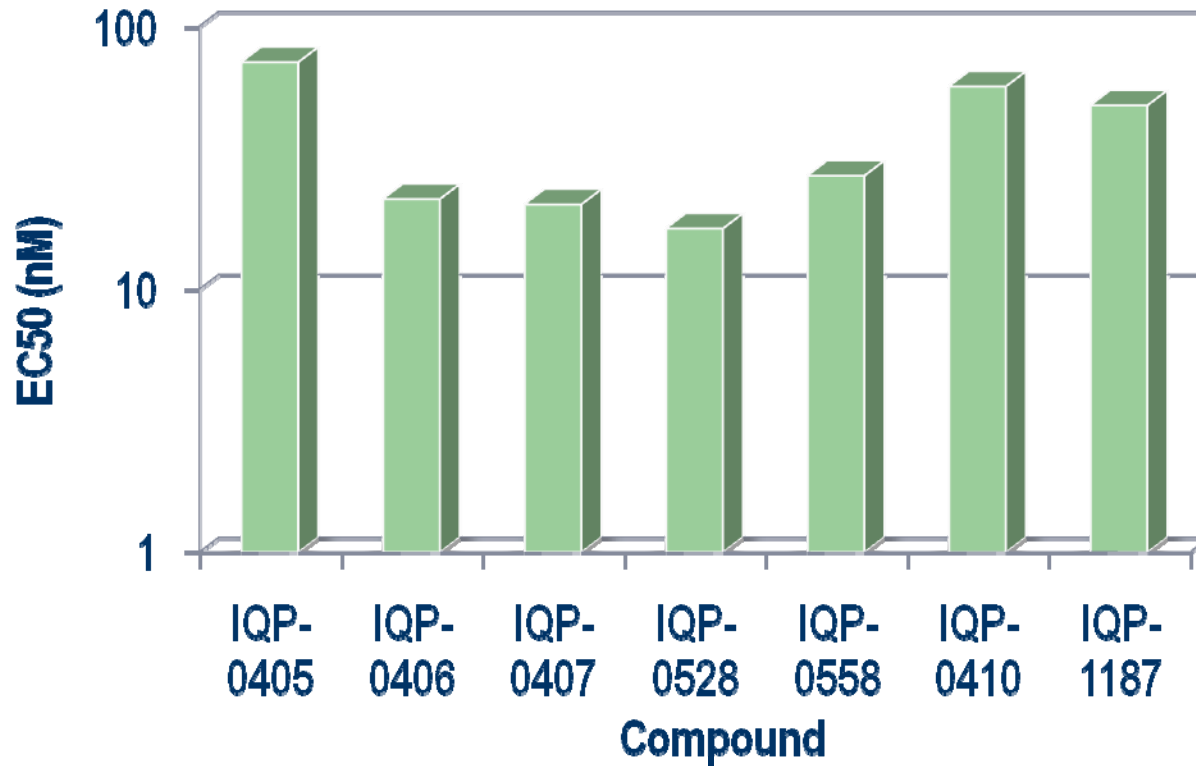


Single Cycle Replication Inhibition Assay Demonstrates IQP-0410 Acts as an HIV-1 RT Inhibitor



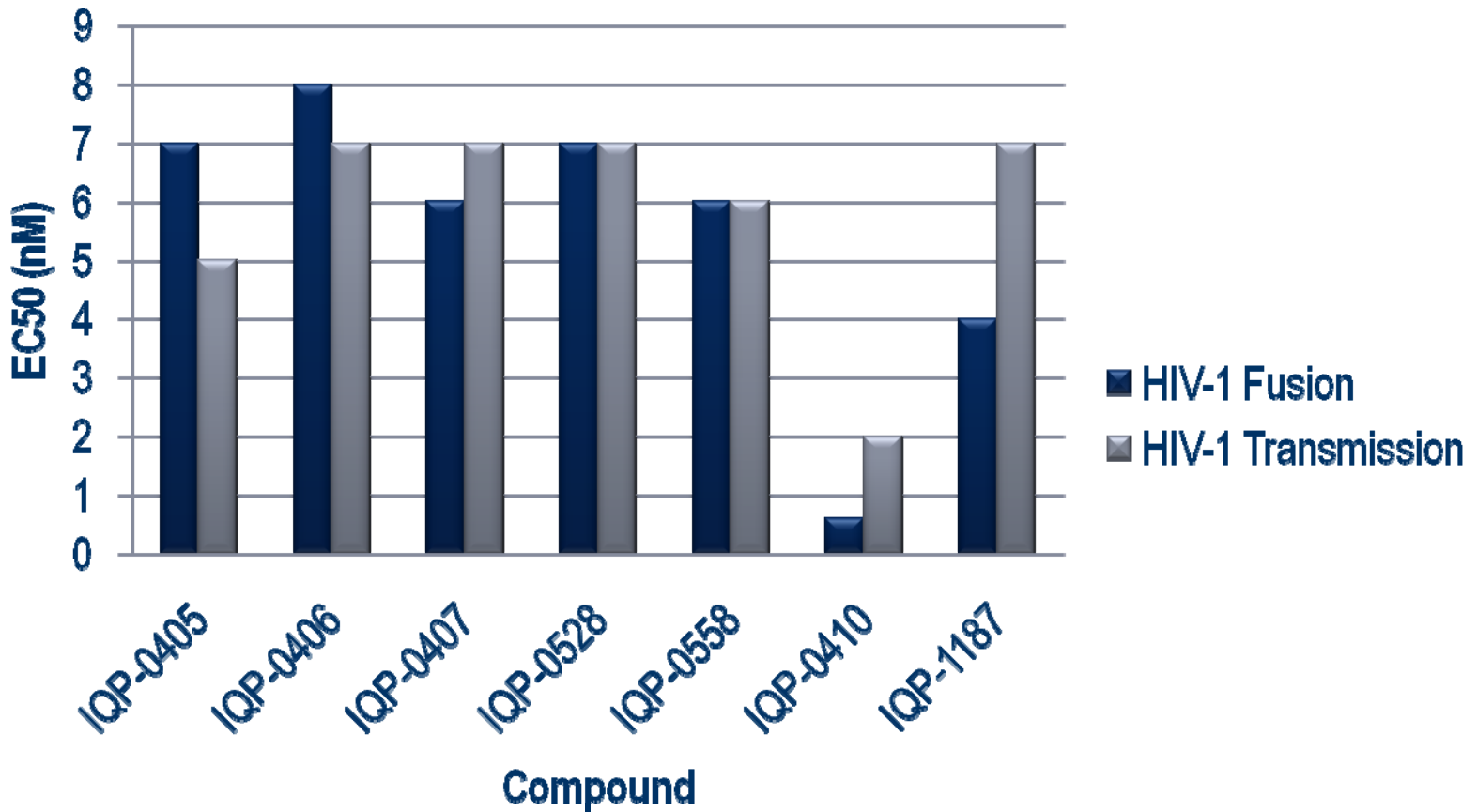
Inhibition of HIV-1_{III B} Entry in MAGI Assay

MAGI cells were incubated with test compound prior to the addition of virus. Infection proceeds for one hour prior to extensive washing to remove all unbound virus and extracellular compound. The endpoint of the assay is performed by quantification of β -galactosidase at 48 hours post-infection.

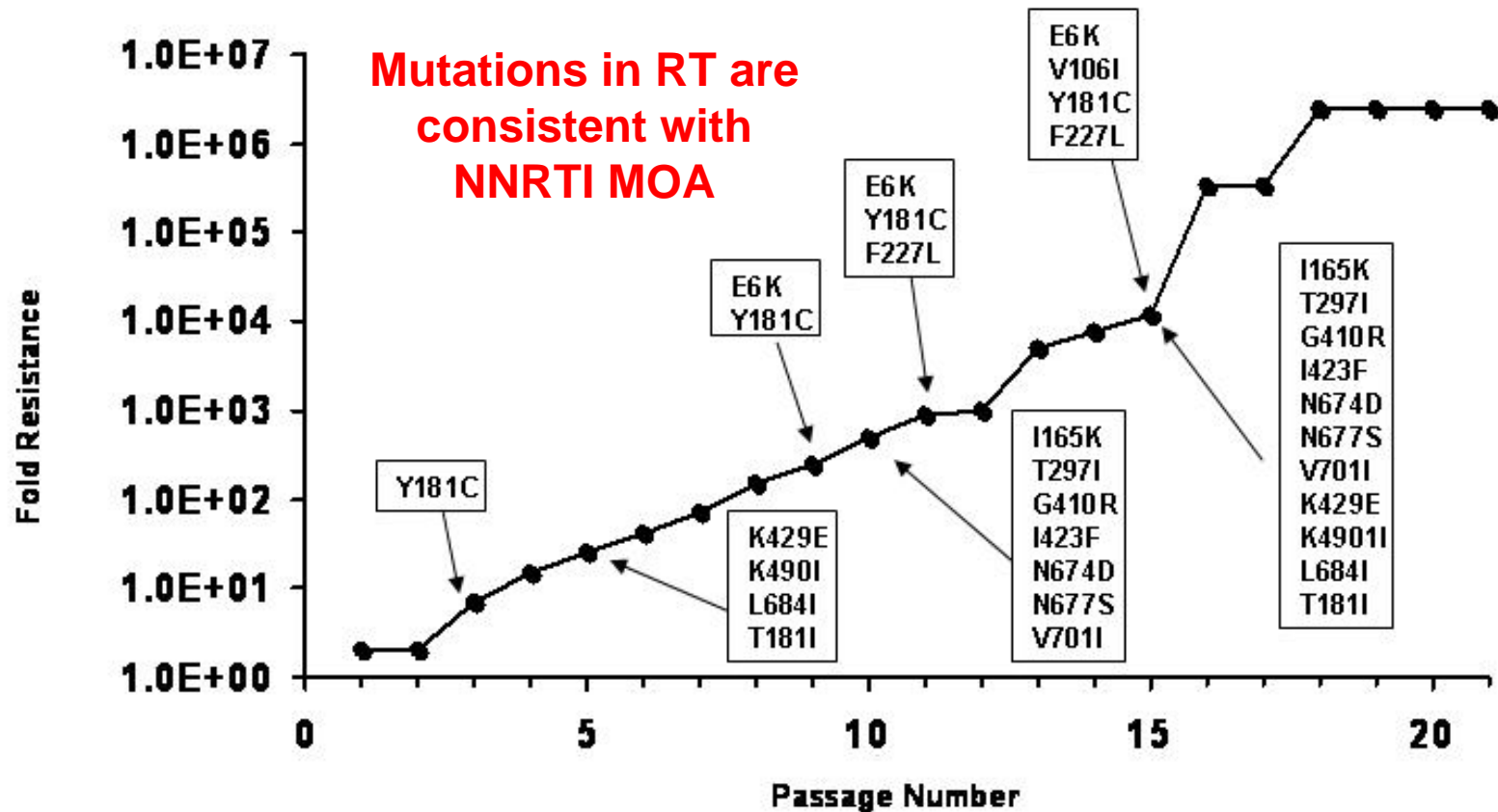


Inhibition of Fusion and Cell-to-Cell Virus Transmission

CEM-SS cells were co-cultured with chronically HIV-infected CEM-SS cells. Syncytium formation was quantified at 48 hours to measure inhibition of fusion. Virus replication was measured by RT assay at 48 hours to quantify the rapid burst of HIV resulting from cell-to-cell virus transmission.

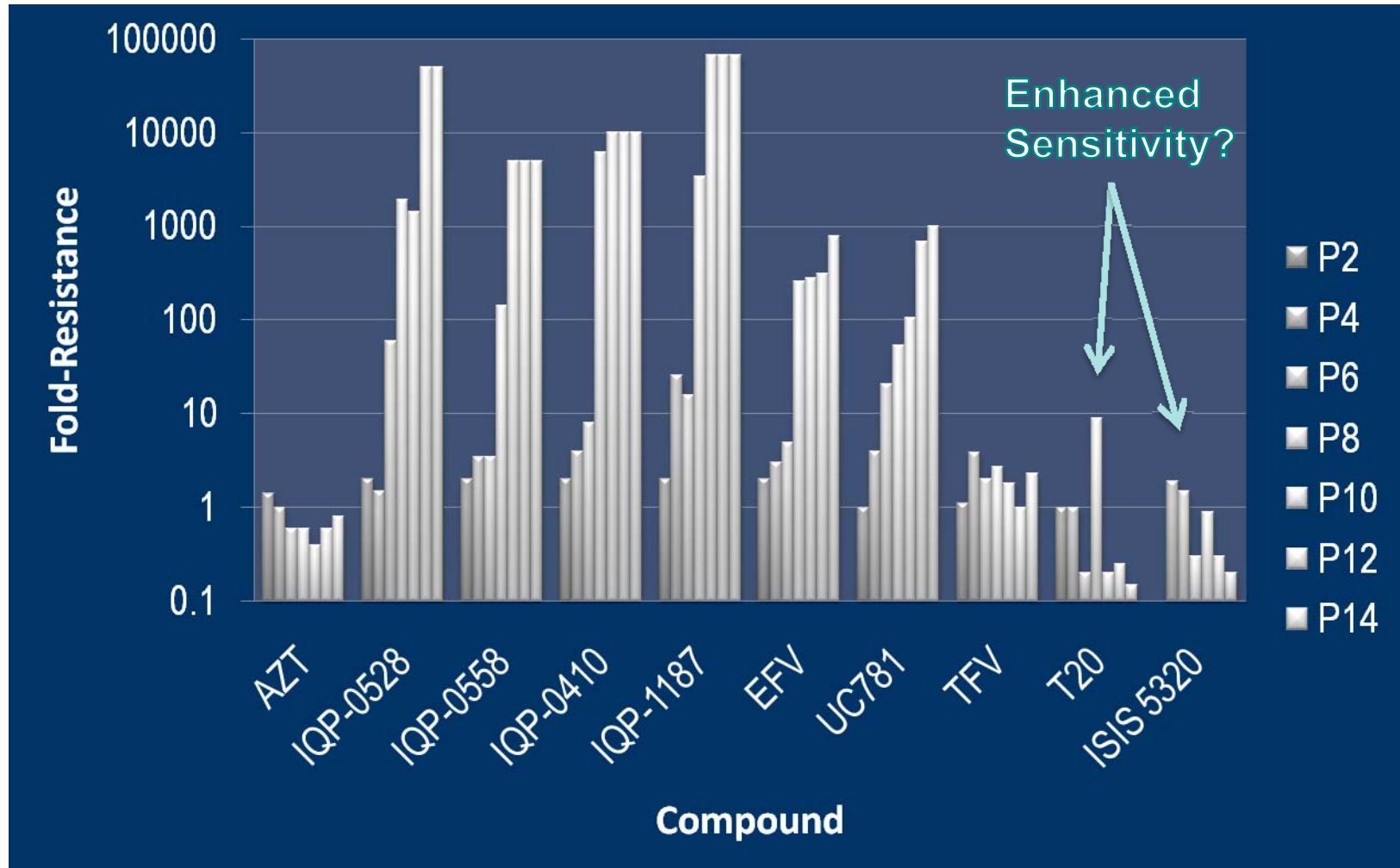


HIV-1 Resistance to IQP-0410



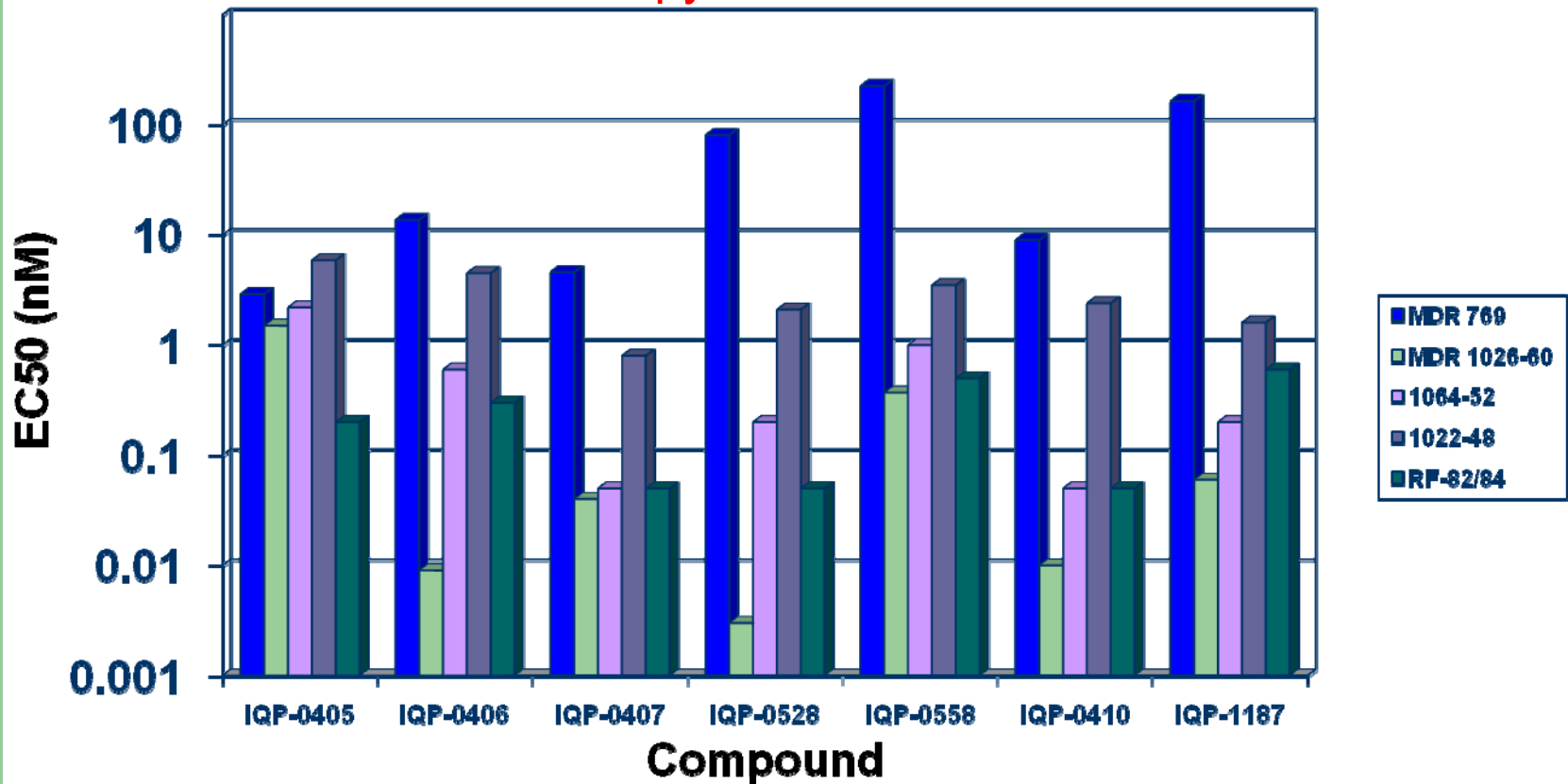
Mutations in gp120 and gp41 are consistent with chemokine receptor engagement and fusion

Cross-Resistance of Inhibitors Against IQP-0410-Resistant Virus



Inhibition of MDR Viruses: Enhanced Activity Against MDR-PI viruses

Increasing mutations in the protease yields increased sensitivity to the pyrimidinediones.

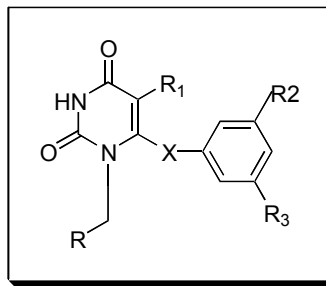


MDR 769: NRTI, NNRTI and PI-resistant;
MDR 1026, MDR 1064, MDR 1022, and RF-82/84: PI-resistant

Preclinical and Clinical Development

- IQP-0410 has completed all IND-directed studies with planned submission to the FDA in late December 2008.
- IQP-0528 being jointly developed as a vaginal topical microbicide with International Partnership for Microbicides (IPM) and CONRAD; anticipated human clinical trials in 2010.
- Next generation pyrimidinedione development is focused on the definition and preclinical evaluation of compounds with a higher barrier to resistance.

Selection of Pyrimidinediones with Higher Genetic Barrier to Resistance



Compound Description			Compound Efficacy		Fold-Resistance to Virus Strains Selected to NNRTIs (IIIB)				Fold-Resistance to Mutagenized Strains with Single Amino Acid Changes in RT (NL4/3)				Fold-Resistance in Biochemical RT Inhibition Assay	
Compound	R	X	WT HIV-1 (IIIB)	TI	Y181C	K103N	L100I	Y181C + K103N	Y181C	K103N	L100I	F227L	K103N	L100I
IQP-0405	Cyclopropyl	O	6	214,954	43	170	5	774	66	46	2	30	8	6
IQP-0406	Cyclopropyl	O	2	15,544	20	23	2	113	33	29	1	1	43	1
IQP-0407	Cyclopropyl	C=O	9	125,447	59	125	2	133	23	3	1	3	25	1
IQP-0528	Cyclopropyl	C=O	0.5	2,551,345	114	66	2	52	10	21	1	1	55	1
IQP-0529	Cyclobutyl	S	12	80,806	5	33	3	15	7	16	2	4	89	4
IQP-0531	Cyclobutyl	O	22	54,244	8	23	5	56	10	33	1	8	8	7
IQP-0547	Phenyl	S	2	598,454	7	25	2	36	55	8	1	15	94	1
IQP-0548	Phenyl	S	1	164,786	14	9	2	59	35	7	1	9	15	1
IQP-0549	Phenyl	O	5	165,294	3	6	1	29	30	20	3	8	2	1
IQP-0558	1-cyclopenten-1-yl	C=O	0.1	523,333										
IQP-0565	3-cyclopenten-1-yl	O	7	170,090	8	23	2	29	12	9	1	8	2	1
IQP-0410	3-cyclopenten-1-yl	C=O	2	553,421	29	21	2	526	24	1	4	2	17	1
IQP-1187	3-cyclopenten-1-yl	C=O	2	491,237	32	138	4	361	177	4	3	3	152	6

Pyrimidinediones with significant activity against NNRTI-resistant viruses:

R substituent: phenyl>butyl>propyl>3-cyclopenten-1-yl

X substituent: O>C=O>S

Compounds IQP-0547, IQP-0548 and IQP-0549 represent new leads for the development of a next generation pyrimidinedione with high genetic barrier to resistance.

Summary: Continued Development of Pyrimidinedione NNRTIs

- The pyrimidinediones inhibit HIV-1 reverse transcriptase but are inactive against HIV-2 reverse transcriptase, typical of NNRTIs. Resistance selection confirms the appearance of NNRTI type mutations in HIV-1 RT (no mutations in HIV-2 RT).
- The pyrimidinediones inhibit HIV-1 and HIV-2 entry to target cells and inhibit the fusion of HIV infected and uninfected cells and cell-to-cell transmission of virus. Resistance mutations are detected in gp120 and gp41 (entry inhibition) as well as in matrix proteins (resistance or fitness mutations?).
- The pyrimidinediones inhibit entry of HIV-1 and HIV-2 to target cells by recognition of a conformational target formed after virus attachment but prior to fusion of the viral and cellular membranes. Resistant viruses show decreased ability to enter cells.
- Dual mechanism of action allows for only a small loss of potency with NNRTI-resistant viruses (50-100-fold) and equivalent to enhanced potency against multi-drug PI-resistant viruses. Potential enhanced sensitivity of resistant viruses to T20 and ISIS 5320?
- Analogs have been identified with enhanced ability to inhibit NNRTI-resistant virus strains. SAR development is in progress to define even more active inhibitors of problematic NNRTI-resistant viruses with lead compounds IQP-0547, 0548 and 0549.

Next Steps in Pyrimidinedione Development

- SAR development based on initial findings.
- Continued evaluation of sensitivity to other NNRTI resistance engendering mutations.
- Selection and characterization of resistant virus strains.
- Rational development of combination therapeutic strategies to further minimize resistance selection potential.
- Direct comparison to Sustiva and new Idenix and Tibotech NNRTIs: differ by dual MOA, enhanced efficacy against MDR strains, lower toxicity; overlapping mutations Y181C.

Acknowledgements

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