

Advanced Development of Dual-Acting Pyrimidinediones as Highly Potent Anti-HIV Therapeutic Drugs and Topical Microbicides

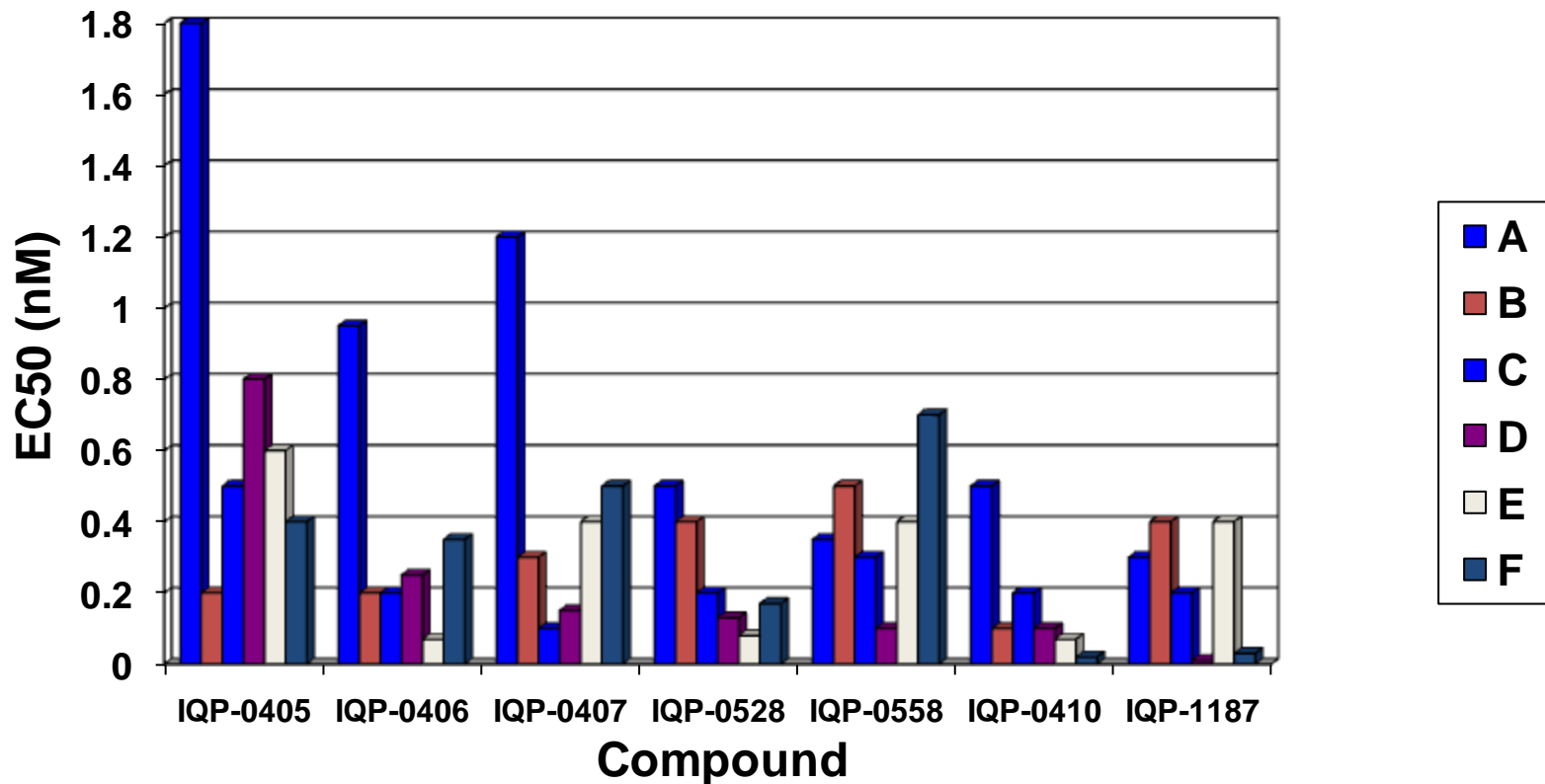
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PYRIMIDINEDIONE THERAPEUTIC DRUG DEVELOPMENT IQP-0410

Antiviral Profile of the Pyrimidinediones

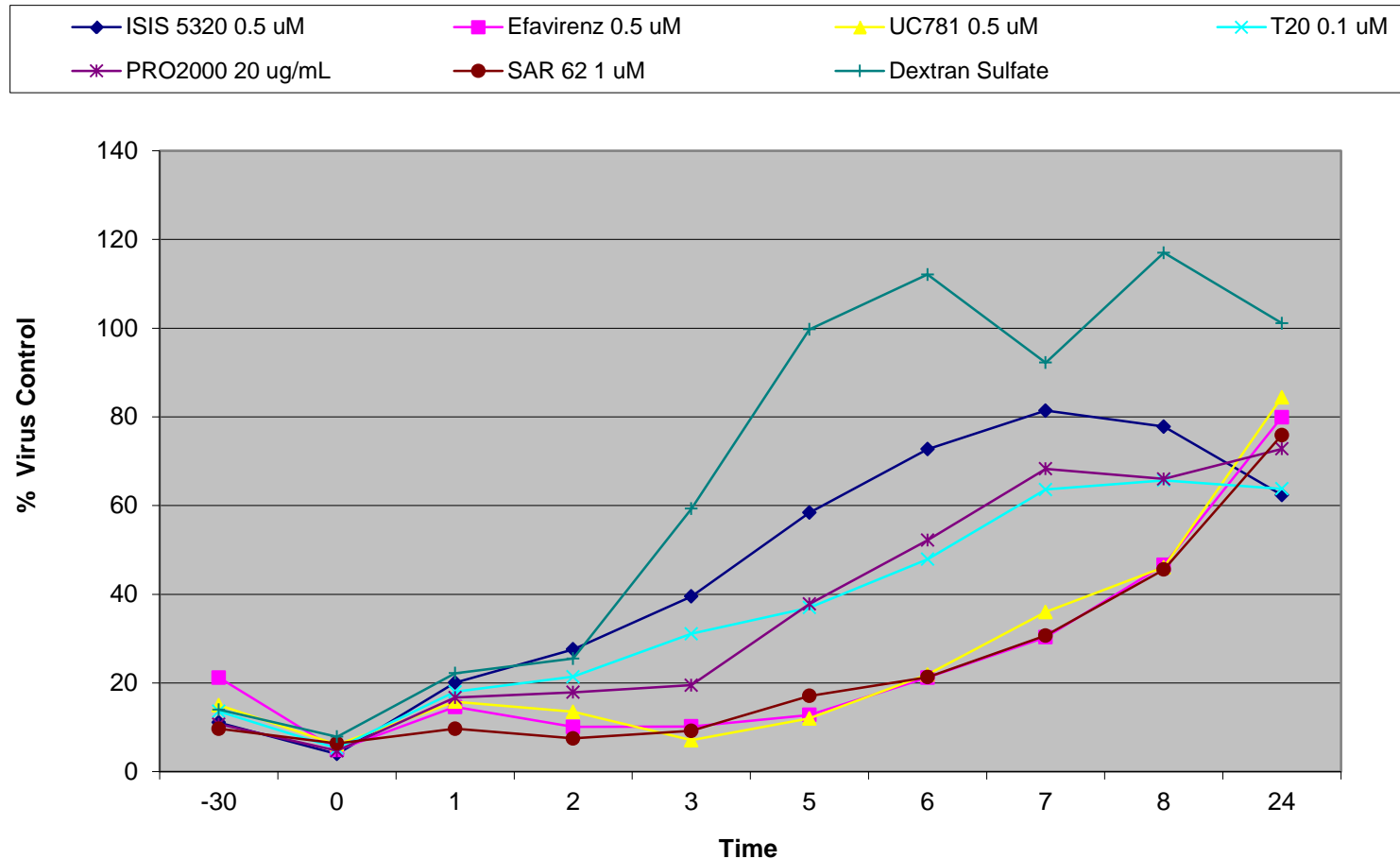
- Highly potent inhibitors of HIV-1 with activity at sub-nanomolar concentration levels and TI values greater than one million.
- Slight (~10-100-fold) loss of efficacy against NNRTI-resistant HIV-1.
- Active against HIV-2 at nanomolar concentration levels with TIs > 100,000.
- Primary mechanism of action: nonnucleoside inhibitor of HIV-1 reverse transcriptase; no activity versus HIV-2 reverse transcriptase.
- Secondary mechanism of action: 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.*

Inhibition of Clinical HIV-1 Subtype Strains



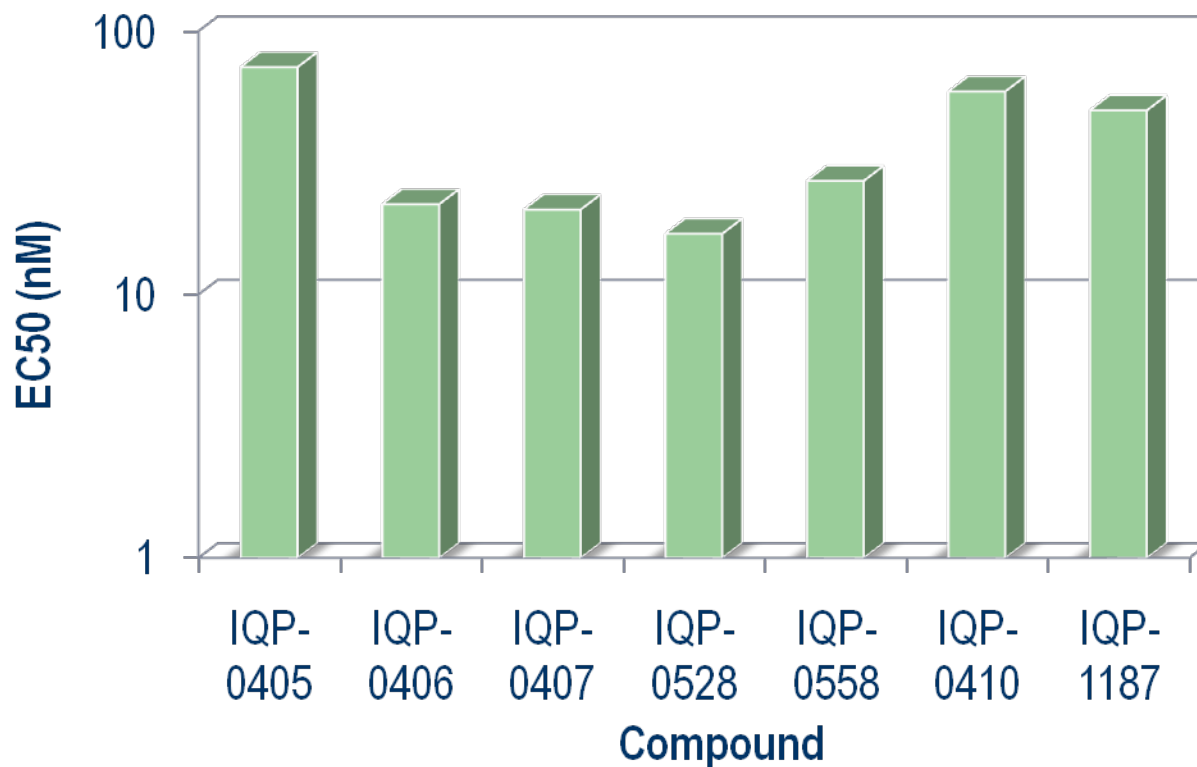
The pyrimidinediones inhibit HIV-1 at sub-nanomolar concentration levels with therapeutic indices of ~1 million

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



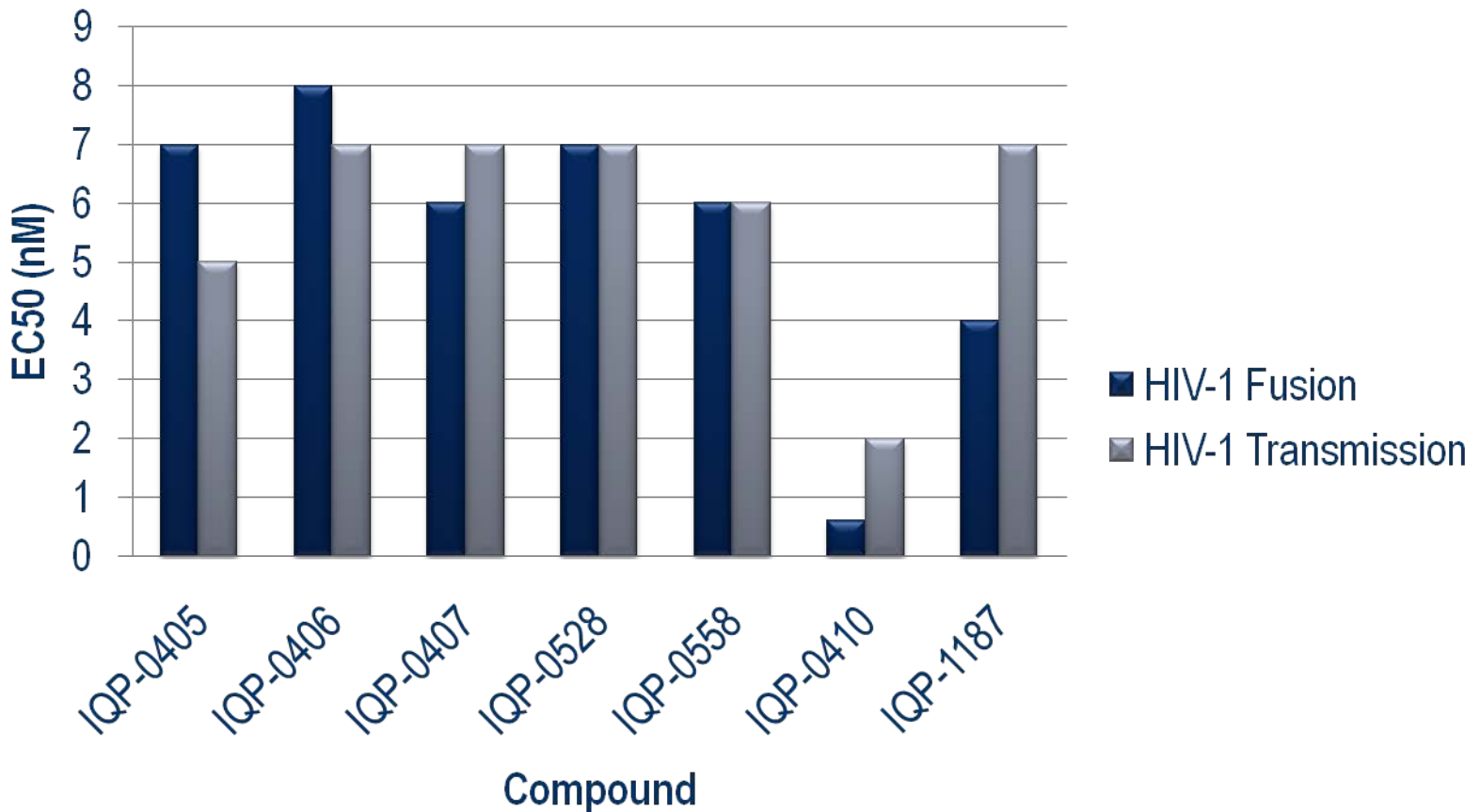
Inhibition of HIV Entry to Target Cells

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.

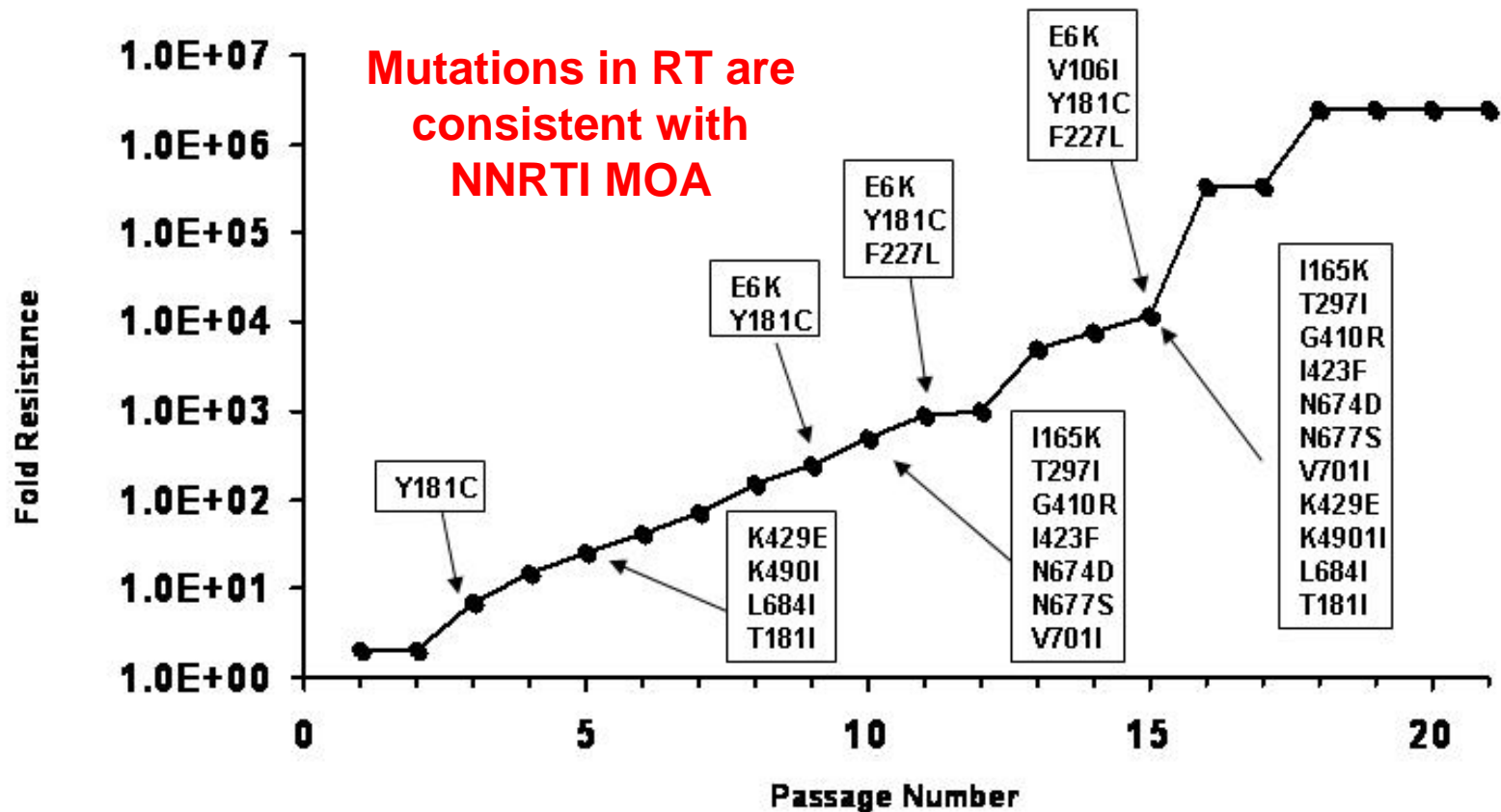


Inhibition of Fusion and Cell-to-Cell Virus Transmission

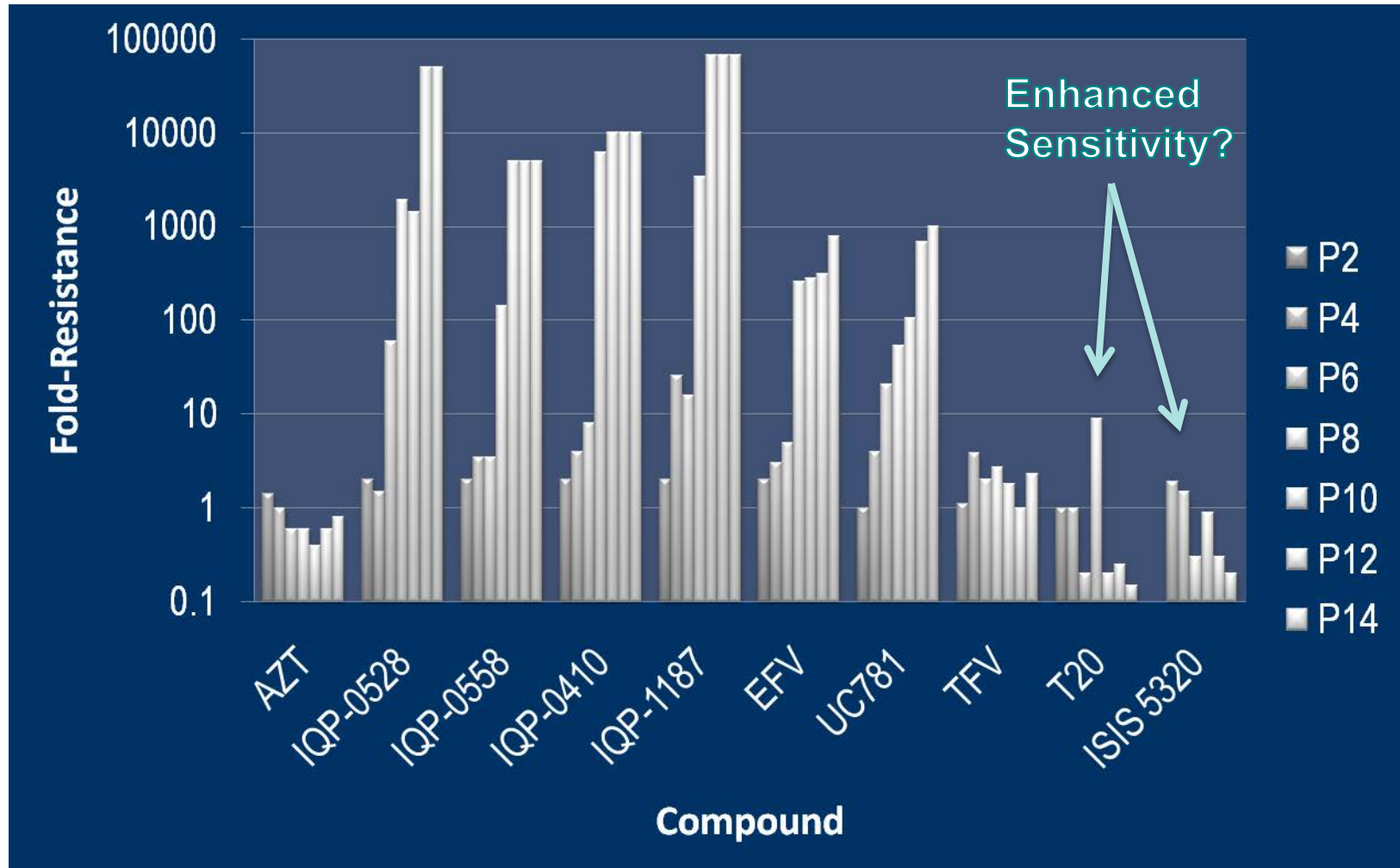
CEM-SS cells were co-cultured with chronically HIV-infected CEM-SS cells. Syncytium formation was quantified at 48 hours to measure inhibition of fusion. Virus replication was measured by RT assay at 48 hours to quantify the rapid burst of HIV resulting from cell-to-cell virus transmission.



HIV-1 Resistance to IQP-0410

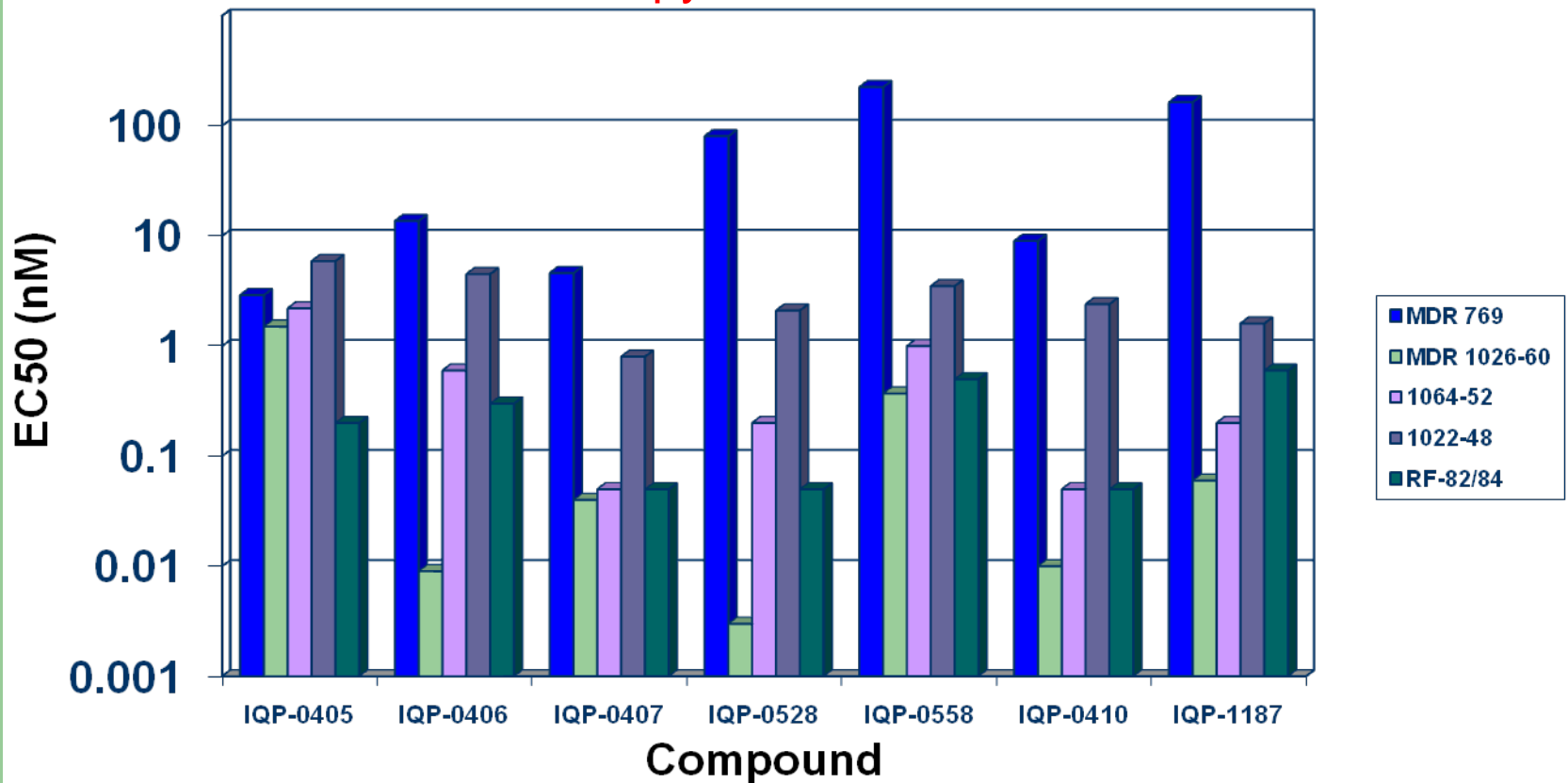


Cross-Resistance of Inhibitors Against IQP-0410-Resistant Virus



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

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



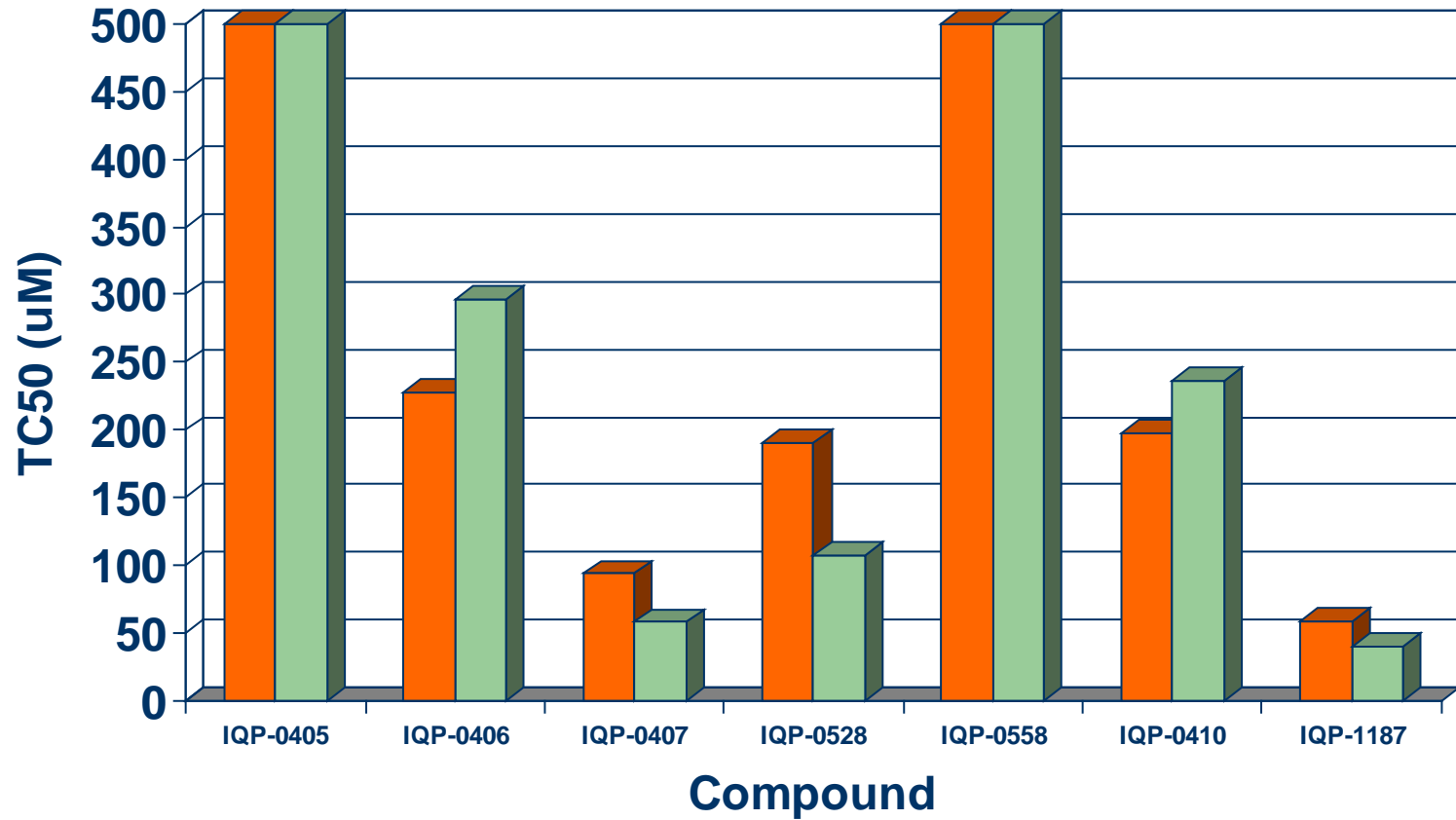
MDR 769: NRTI, NNRTI and PI-resistant;
MDR 1026, MDR 1064, MDR 1022, and RF-82/84: PI-resistant

IQP-0410 Mechanism of Action

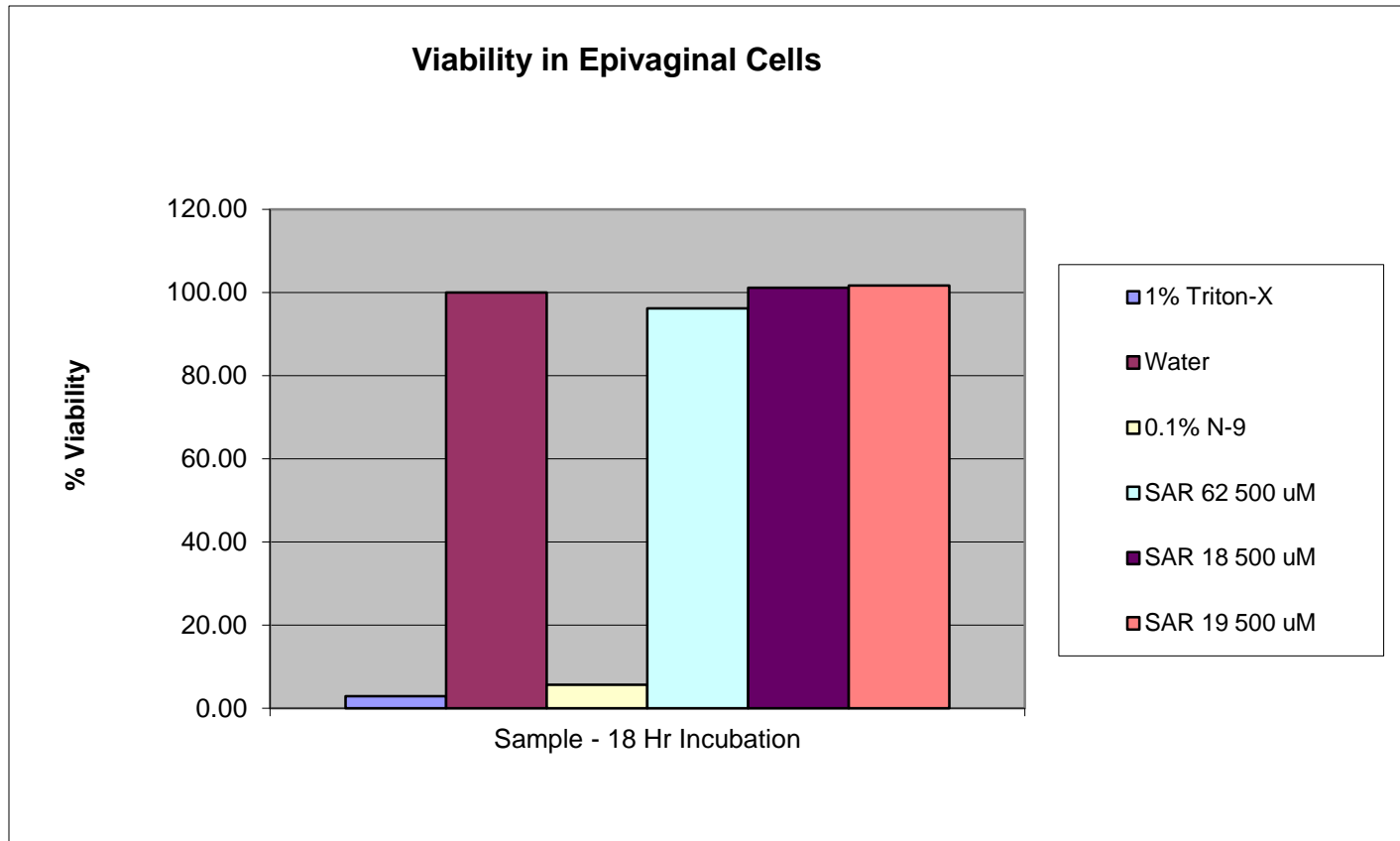
- IQP-0410 inhibits HIV-1 reverse transcriptase ($K_i = 3.2 \text{ nM}$) but is inactive against HIV-2 reverse transcriptase, typical of NNRTIs. **Resistance selection confirms the appearance of NNRTI type mutations in HIV-1 RT (no mutations in HIV-2 RT).**
- IQP-0410 inhibits HIV-1 and HIV-2 entry to target cells and inhibits the fusion of HIV infected and uninfected cells and cell-to-cell transmission of virus. **Resistance mutations detected in gp120 and gp41 (entry inhibition).**
- IQP-0410 inhibits 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.**
- IQP-0410 is not virucidal.
- IQP-0410 does not directly interfere with gp120-CD4 interaction although the presence of CD4 on the target cell is necessary.
- Dual mechanism allows for only small loss of potency with NNRTI-resistant viruses and equal to enhanced potency against PI-resistant viruses. **Enhanced sensitivity of IQP-0410-resistant viruses to T20 and ISIS 5320 observed.**

**PYRIMIDINEDIONE
TOPICAL MICROBICIDE DEVELOPMENT
IQP-0528**

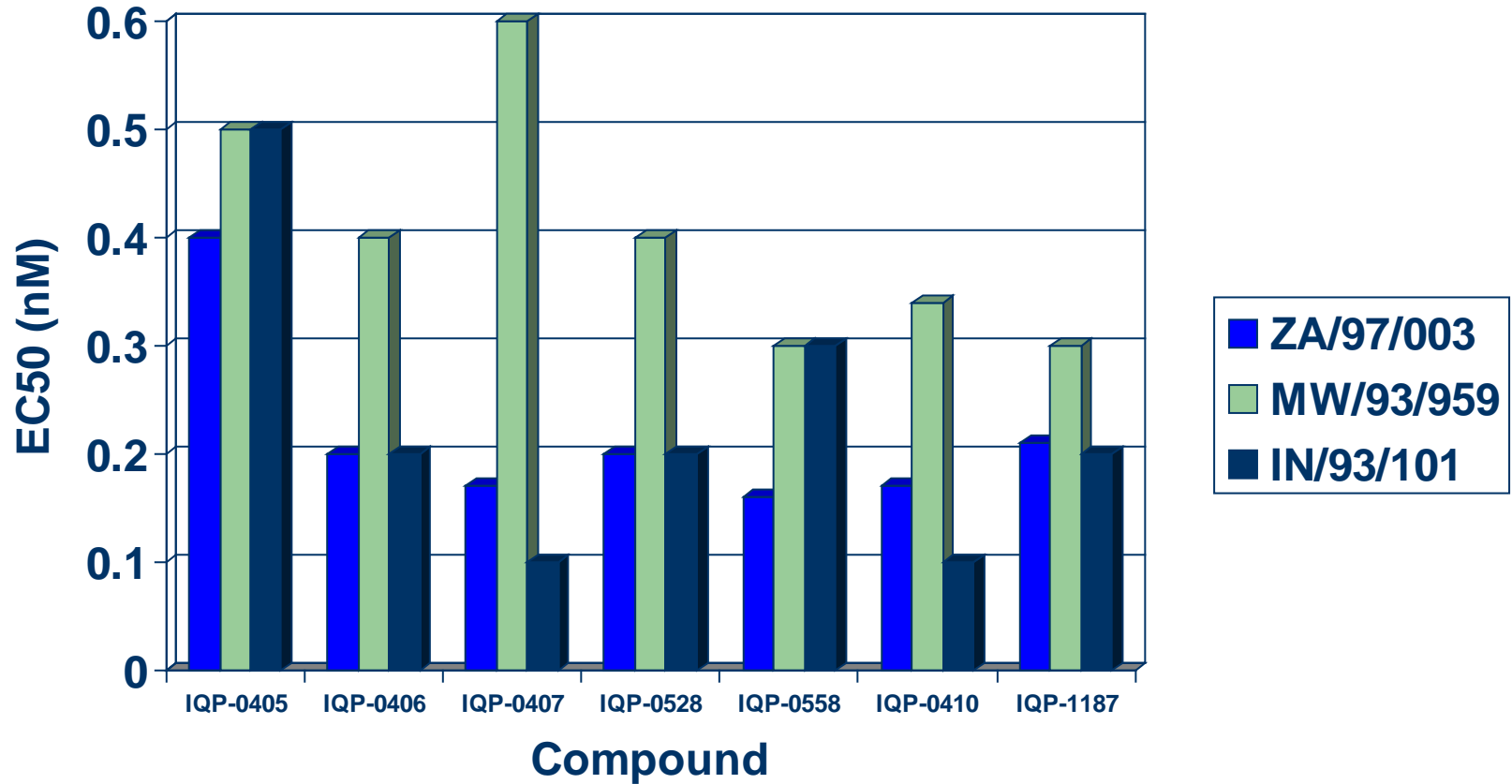
Toxicity to *Lactobacilli*



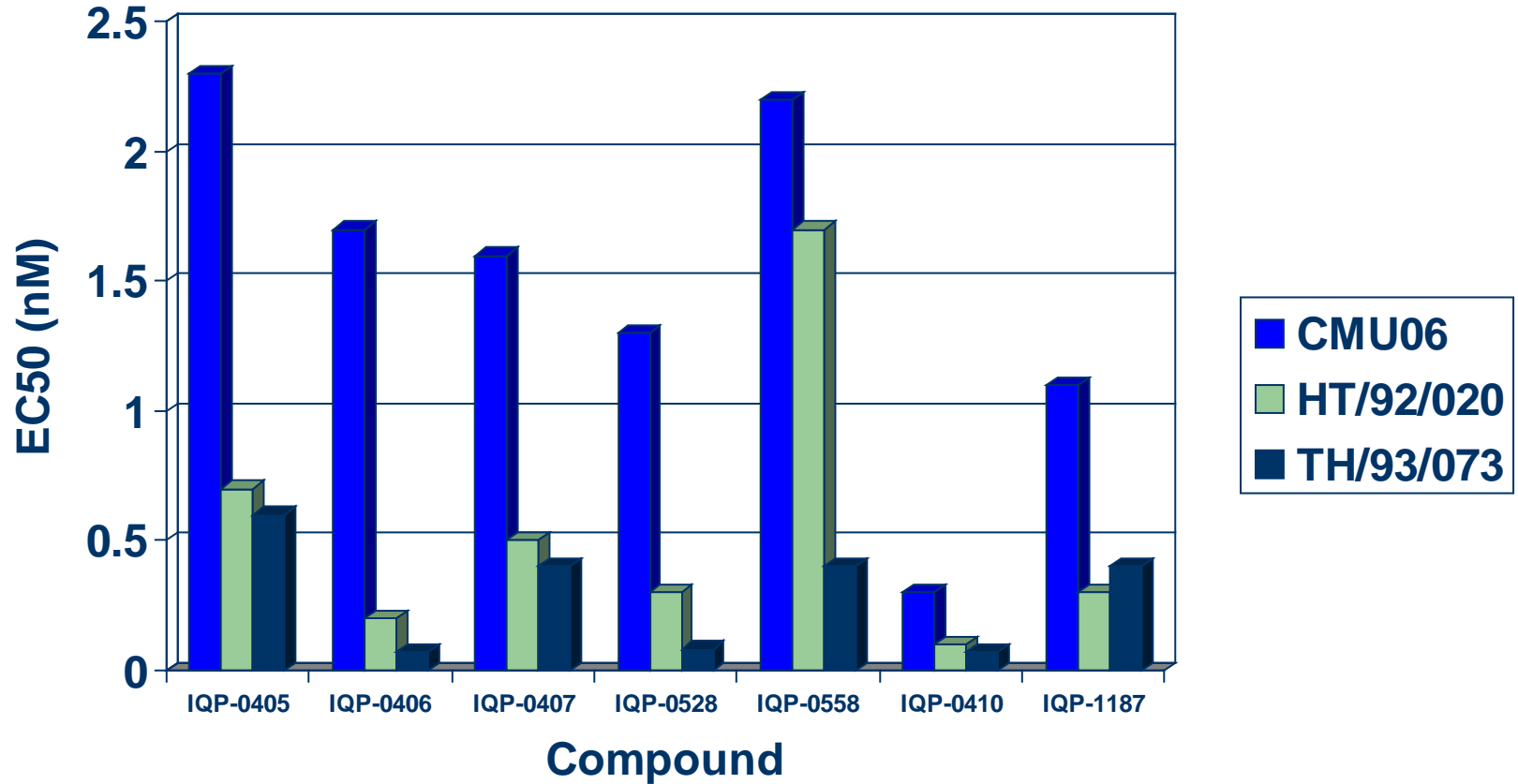
Toxicity of Pyrimidinediones to MatTek Epivaginal Tissue



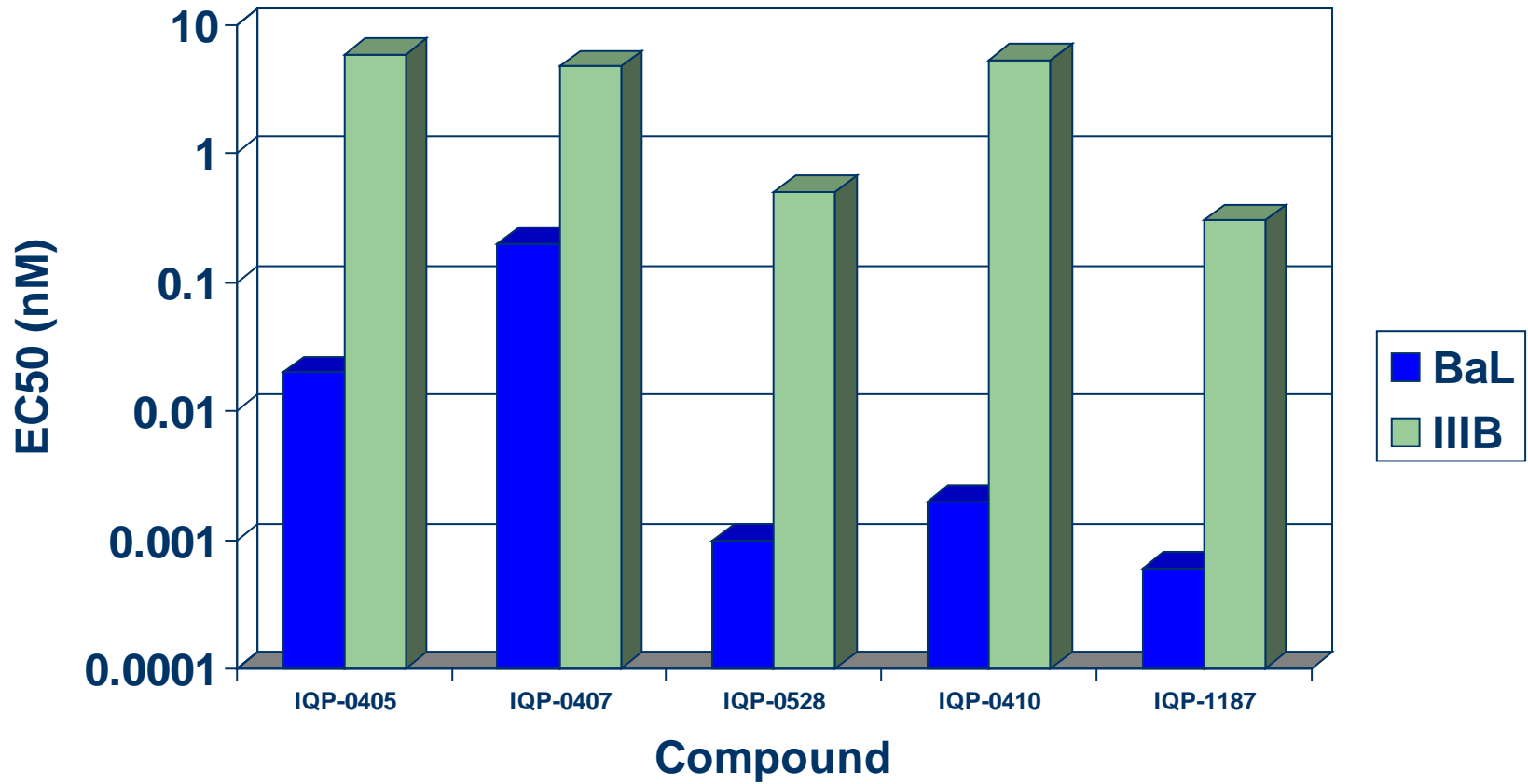
Inhibition of Subtype C Viruses



Inhibition of Subtype E Viruses



Efficacy in Cervical Explant Model



Conclusions

- Pyrimidinediones represent a highly attractive chemotype for microbicide development:
 - High potency against clinical virus strains
 - Low toxicity to human cells and *Lactobacilli*
 - Dual mechanism of action at steps relevant for microbicide action: entry and RT inhibition
 - High potency against drug resistant and multi-drug resistant virus strains
 - Remain active in the presence of semen and vaginal fluids and at pH 4
- Attractive candidates for combination therapy:
 - Combination product with other microbicide agents to provide multi-target microbicide barrier
 - Combination product with two members of the pyrimidinedione class (potent RT inhibitor plus potent entry inhibitor)

Acknowledgements

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